REGISTRATION REPORT

Part B

Section 6

Mammalian Toxicology

Detailed summary of the risk assessment

Product code: GLOB1310aH

Product name(s): Glosset Ace

Chemical active substance(s):

Aclonifen, 540 g/L

Flufenacet, 60g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Globachem NV

Submission date: December 2021

MS Finalisation date: 25/08/2022

After commenting: 14/12/2022 /corrected 14/04/2023 / Revised 21/04/2023

Version history

When	What
December 2021	Initial submission by the applicant for approval of new product.
August 2022	First zRMS PL evaluation
December 2022	Corrections made by zRMS PL after commenting round
April 2023	Correction/Revision made by zRMS

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6 Mammalian Toxicology (KCP 7)

6.1 Summary

Table 0-1: Information on GLOB1310aH/Glosset Ace *

Product name and code	GLOB1310aH/Glosset Ace
Formulation type	Suspension concentrate [SC]
Active substance(s) (incl. content)	Aclonifen 540g/L Flufenacet 60 g/L
Function	Herbicide
Product already evaluated as the 'representative formulation' during the approval of the active substance(s)	No
Product previously evaluated in another MS according to Uniform Principles	No

* Information on the detailed composition of GLOB1310aH/Glosset Ace can be found in the confidential dRR Part C.

Justified proposals for classification and labelling

According to the criteria given in Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008, the following classification and labelling with regard to toxicological data is proposed for the preparation:

Table 0-2: Justified proposals for classification and labelling for GLOB1310aH/Glosset Ace according to Regulation (EC) No 1272/2008 – Health Hazard

Hazard class(es), categories	Skin Sens. 1 Carc. 2
Hazard pictograms or Code(s) for hazard pictogram(s)	GHS07 GHS08
Signal word	Warning
Hazard statement(s)	H317: May cause an allergic skin reaction H351: Suspected of causing cancer
Precautionary statement(s)	P201, P202, **P261,** P272, **P280**, **P302+P352**, P308 + P313**, P333+P313**, **P362+P364**, P405, ~~**P501**~~
Additional labelling phrases	To avoid risks to man and the environment, comply with the instructions for use. [EUH401]
Contains:	aclonifen, flufenacet, 1,2-benzisothiazol-3(2H)-one ~~(CAS no. 2634-33-5) may produce an allergic reaction. [EUH208]~~

P statements **in bold** represents "highly recommended" and "recommended" statements according to GD on application of the CLP criteria (Version 5.0 July 2017).

Table 0-3: Summary of risk assessment for operators, workers, residents and bystanders for GLOB1310aH/Glosset Ace

	Result	PPE / Risk mitigation measures
Operators	Acceptable	PPE (based on estimation of exposure – table 6.6-3)- Gloves during mixing/loading (work wear – arms, body and legs covered) Taking into account CLP classiffication of the formulation (Sin Sens. 1 and Carc. 2) GLOB1310aH/Glosset Ace and in-use dilutions (Skin Sens. 1) the operator should wear protective clothing, protective gloves and face/eye protection during mixing, loading and application.
Workers	Acceptable	None - No PPE (work wear – arms, body and legs covered) ~~Treated crops should not be re-entered before spray deposits on leaf surfaces have completely dried~~[1].
Residents	Acceptable	~~None~~ ≥ 50% drift-reducing nozzles are highly recommended (due to skin sensitising properties of spray dilutions)
Bystanders	Acceptable	

No unacceptable risk for operators, workers, residents and bystanders was identified when the product is used as intended and provided that the PPE/ risk mitigation measures stated in P statements **in bold** represents "highly recommended" and "recommended" statements according to GD on application of the CLP criteria (Version 5.0 July 2017).

Table 0-3 are applied.

For operators, due to the classification of the product, it is also recommended to wear protective clothing, eye protection/face protection when handling the product or its dilution.

A summary of the critical uses and the overall conclusion regarding exposure for operators, workers and residents/bystanders is presented in the following table.

Table 0-4 Critical uses and overall conclusion of exposure assessment

1	2	3	4	5	6	7	8	9	10			
Use-No.*	**Crops and situation (e.g. growth stage of crop)**	**F, Fn, Fpn G, Gn, Gpn or I ****	**Application**		**Application rate**		**PHI (d)**	**Remarks:** **(e.g. safener/synergist (L/ha))** **critical gap for operator, worker, resident or bystander exposure based on [Exposure model]**	**Acceptability of exposure assessment**			
			Method / Kind **(incl. application technique *****	**Max. number (min. interval between applications)** **a) per use** **b) per crop/ season**	**Max. application rate kg as/ha** **a) a.s. 1** **b) a.s. 2**	**Water L/ha** **min / max**			**Operator**	**Worker**	**Residents**	**Bystander**
1-6	Winter cereals BBCH 00-09 (Sep-Dec)	F	Downwards spraying	1 ; 1	a)0.810 kg Aclonifen/ha b) 0.090 kg Flufenacet/ha	150 - 300	Not relevant	Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products; EFSA Journal 2014;12(10):3874	R	A	A	A
7-12	Winter cereals BBCH 00-09 (Sep-Dec)	F	Downwards spraying	1 ; 1	a)1.08 kg Aclonifen/ha b) 0.12 kg Flufenacet/ha	200 - 300	Not relevant		R	A	A	A

[1] The statement is not relevant due to the absence of foliage, exposure of workers towards dislodgeable foliar residues when bare soil is treated with the product (germination stage of crop – BBCH 00-09).

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1
** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
*** e.g. LC: low crops, HC: high crop, TM: tractor-mounted, HH: hand-held

Explanation for column 10 "Acceptability of exposure assessment"

A	Exposure acceptable without PPE / risk mitigation measures
R	Further refinement and/or risk mitigation measures required
N	Exposure not acceptable/ Evaluation not possible

Data gaps

Noticed data gaps are: none

6.2 Toxicological Information on Active Substances

Information regarding classification of the active substances and on EU endpoints and critical areas of concern identified during the EU review are given in Table 0-1.

Table 0-1: Information on active substances

	Aclonifen	**Flufenacet**
Common Name	Aclonifen	Flufenacet
CAS-No.	74070-46-5	142459-58-3
Classification and proposed labelling		
With regard to toxicological endpoints (according to the criteria in Reg. 1272/2008, as amended))	Hazard classes (s), categories[1)]: Skin Sens. 1A, Carc. 2 Code(s) for hazard pictogram(s): GSH07, GHS08 Signal word: Warning Hazard statement(s): H317, H351 ~~Precautionary statement(s):~~ ~~P273~~ ~~P280,~~ ~~P308+P313~~	Hazard classes, categories[1)]: Acute Tox. 4* (ORAL) Skin Sens. 1 STOT RE 2* Code(s) for hazard pictogram(s): GHS07 ~~GHS09~~ GHS08 Signal word: Warning Hazard statement(s): H302 H317 H373 ~~Precautionary statement(s):~~ ~~P273~~ ~~P280~~ ~~P308+P311~~
Additional C&L proposal	/	/
Agreed EU endpoints		
AOEL systemic	0.07 mg/kg bw/d (Oral absorption: 100%)	0.017 mg/kg bw/d (Oral absorption: 100%)
Reference	EFSA Conclusion (2008), 149, 1 – 80 SANCO/161/08 – rev. 2, 2012	EU Review report (7469/VI/98-Final – 3rd July 2003)

	Aclonifen	Flufenacet
Conditions to take into account/critical areas of concern with regard to toxicology		
According to Review Report/EFSA Conclusion for active substance	Member states should pay attention to the protection of the operators safety. Authorised conditions of use must prescribe the application of adequate personal protective equipment and risk mitigation measures to reduce the exposure.	Member states should pay attention to the protection of operators. Risk mitigation measures should be applied where appropriate

[1)] Harmonised classification - Annex VI of Regulation (EC) No 1272/2008 (CLP Regulation)
* Minimum classification

6.3 Toxicological Evaluation of Plant Protection Product

A summary of the toxicological evaluation for GLOB1310aH/Glosset Ace is given in the following tables. No tests were performed on product GLOB1310aH. For the acute oral toxicity, acute dermal toxicity, skin and eye irritation and skin sensitisation the assessment has been conducted according to Regulation EC 1272/2008. Full details on composition and classification of formulants are provided in part C, of this registration report.
Table 6.3-1 shows the classification of GLOB1310aH based on theoretical calculation and toxicity data for its components.

Table 0-1: Summary of evaluation of the studies on acute toxicity including irritancy and skin sensitisation for GLOB1310aH/Glosset Ace

Type of test, species, model system (Guideline)	Result	Acceptability	Classification (acc. to the criteria in Reg. 1272/2008)	Reference
LD_{50} oral, rat (OECD 423)	Study not necessary.	Yes	None	Theoretical calculations (see Part C)
LD_{50} dermal, rat (OECD 402)	Study not necessary.	Yes	None	Theoretical calculations (see Part C)
LC_{50} inhalation, rat (OECD 403)	Study not necessary.	Yes	None	Theoretical calculations (see Part C)
Skin irritation, (OECD 404)	Study not necessary.	Yes	None	Theoretical calculations (see Part C)
Eye irritation, (OECD 405)	Study not necessary.	Yes	None	Theoretical calculations (see Part C)
Skin sensitisation	Study not necessary	Yes	Skin. Sens. 1, H317	Theoretical calculations (see Part C)
Supplementary studies for combinations of plant protection products	No data – not required	/	/	/

Table 0-2: Additional toxicological information relevant for classification/labelling of GLOB1310aH/Glosset Ace

	Substance (concentration in product, % w/w)	**Classification of the substance (acc. to the criteria in Reg. 1272/2008)**	**Reference**	**Classification of product (acc. to the criteria in Reg. 1272/2008)**
Toxicological properties of active substance(s) (relevant for classification of product)	Aclonifen 44.86% w/w	Skin Sens. 1A, H317 (>0.1%) Carc. 2, H351 (>1%)	Reg. 1272/2008 / MSDS**	Skin. Sens. 1, H317 Carc. 2, H351
	Flufenacet 5.15% w/w	Acute Tox. 4*, H302 (calculated ATE_{mix}>2000, not relevant) Skin Sens. 1, H317 (>1%) STOT RE 2*, H373 (<10%, not relevant)	Reg. 1272/2008 / MSDS**	Skin. Sens. 1, H317
Toxicological properties of non-active substance(s) (relevant for classification of product)	1,2-benzisothiozolin-3-one, CAS no.: 2634-33-5; <0.05% w/w	Acute Tox. 4; H302 (not relevant for the product) Skin Irrit. 2; H315 (not relevant for the product) Eye Dam. 1; H318 (not relevant for the product) Skin Sens. 1; H317, SCL 0.05% (<SCL=0.05% and > elicitation limit=0.005%)	Reg. 1272/2008 / MSDS**	Phrase on the label: "Contains 1,2-Benzisothiazolin-3-one"
Further toxicological information	No data – not required			Contains 1,2-benzisothia-zol-3(2H)-one, may produce an allergic reaction" (see Part C for remarks)

** Material safety data sheet by the applicant

6.4 Toxicological Evaluation of Groundwater Metabolites

Aclonifen:
There are no metabolites in PECgw originated from the use of Aclonifen.

Flufenacet:

The following data on metabolites with the potential to reach the groundwater in concentrations above 0.1 µg/L (FOE-sulfonic acid or M2 and FOE-oxalate or M1) and requiring relevance assessment were submitted. Note that the relevance assessment of the metabolites is reported in Part B.10; the submitted toxicological studies are summarised in this document.

6.4.1 FOE-sulfonic acid (M2)

An overview of the results of the accepted toxicological studies for groundwater metabolite FOE-sulfonic acid (M2) is given in the following table. Full summaries of studies on the metabolite that have not previously been considered within an EU peer review process are described in detail in Appendix 2 (0 Other/Special Studies).

Table 0-1: Summary of the results of toxicity studies for FOE-sulfonic acid

Type of test, species (Guideline)	Result	Acceptability	Reference
Bacterial reverse mutation assay (S. typhimurium) (OECD 471)	non-genotoxic	Yes / No / Supplementary	Herbold, B.A., 2000 (29473)*
Cell gene mutation test (OECD 476)	negative	Yes / No / Supplementary	Vinoth, A., 2019 (19-046-G)
Mammalian cell chromosome aberration test (OECD 473)	negative	Yes / No / Supplementary	

* indicates that a study was reviewed at EU level. This study was submitted in first EU flufenacet review ans was evaluated in the DAR, thus it is not subject to data protection and can be used in the frame of this submission.

6.4.2 FOE-oxalate (M1)

An overview of the results of the accepted toxicological studies for groundwater metabolite FOE-oxalate (M1) is given in the following table. Full summaries of studies on the metabolite that have not previously been considered within an EU peer review process are described in detail in Appendix 2 (0 Other/Special Studies).

Table 0-2: Summary of the results of toxicity studies for FOE-sulfonic acid

Type of test, species (Guideline)	Result	Acceptability	Reference
Bacterial reverse mutation assay (OECD 471)	negative	Yes / No / Supplementary	Savineau , C. 2016a (2015-FRU-1)
Mammalian Cell gene mutation test (OECD 490)	negative	Yes / No / Supplementary	
Mammalian cell chromosome aberration test (OECD 473)	negative	Yes / No / Supplementary	

* indicates that a study was reviewed at EU level

6.5 Dermal Absorption (KCP 7.3)

Proposed dermal absorption rates for Aclonifen are based on a dermal absorption study on [^{14}C]Aclonifen formulated as GLOB1310aH (SC containing 540 g/L of Aclonifen and 60 g/L of Flufenacet). The study results are summarised in the following table. A full summary of this study on the dermal absorption of Aclonifen that has not previously been evaluated within an EU peer review process is provided in 0.

No data on dermal absorption for flufenacet in GLOB1310aH. Justifications for default values according to Guidance on Dermal Absorption (EFSA Journal 2017;15(6):4873) are presented in the following table.

Table 0-1: Dermal absorption rates for active substances in GLOB1310aH/Glosset Ace

	Aclonifen		Flufenacet	
	Value	Reference	Value	Reference
Concentrate	0.043 %	New study reported in 0 (Hassler, S., 2020)	10 %	Default value according to EFSA Guidance on Dermal absorption 2017 (water based formulation category, GLOB1310aH is a SC)
Dilution (1:200)	2.1 %	New study reported in 0 (Hassler, S., 2020)	50 %	Default value according to EFSA Guidance on Dermal absorption 2017 (water based

	Aclonifen		Flufenacet	
	Value	**Reference**	**Value**	**Reference**
				formulation category, GLOB1310aH is a SC)

6.5.1 Justification for proposed values – Aclonifen

Proposed dermal absorption rates for Aclonifen are based on dermal absorption studies on a formulation identical to GLOB1310aH/Glosset Ace. The study results are summarized in the following table. Full summaries of studies on the dermal absorption of Aclonifen/GLOB1310aH that have not previously been evaluated within an EU peer review process are described in detail in 0.

Table 0-2: Summary of the results of submitted dermal absorption study for Aclonifen

Test	Concentrate	Spray dilution (1:200)	Formulation in study	Acceptability of study	Justification provided on representativity of study formulation for current product	Acceptability of justification	Reference*
In vitro (human)	0.043 %	2.1 %	GLOB1310aH	Yes Endpoints can be used for current product.	Not required	Study was performed on formulation GLOB1310aH/Glosset Ace. Justification is not required.	

* indicates that a study was reviewed at EU level

6.5.2 Justification for proposed values – Flufenacet

No data on dermal absorption for flufenacet in GLOB1310aH is available. Justifications for default values for flufenacet according to Guidance on Dermal Absorption (EFSA Journal 2017;15(6):4873) are presented in the following table.

Table 0-3: Default dermal absorption rates for flufenacet in GLOB1310aH (SC)

	Value	Justification for value	Acceptability of justification
Concentrate	10%	Default values for suspension concentrate (SC) / water based formulation category, according to EFSA Guidance on Dermal absorption 2017	Default values proposed by the applicant in the absence of experimental data are accepable
Dilution	50%		

6.6 Exposure Assessment of Plant Protection Product (KCP 7.2)

Table 0-1: Product information and toxicological reference values used for exposure assessment

Product name and code	GLOB1310aH/Glosset Ace	
Formulation type	SC	
Category	Herbicide	
Container size(s), short description	0.25, 0.5, 1, 5, 10, 15, 20 L HDPE, HDPE/PA, HDPE/F and HDPE/EVOH	
Active substance(s) (incl. content)	**Aclonifen** 540 g/L	**Flufenacet** 60 g/L
AOEL systemic	0.07 mg/kg bw/d	0.017 mg/kg bw/d
Inhalation absorption	100 %	100 %
Oral absorption	100 %	100 %
Dermal absorption	Concentrate: 0.043 % Dilution: 2.1 % (Dilution rate: 1:200) (Based on product (GLOB1310aH))	Concentrate: 10 % Dilution: 50 % (Default)

6.6.1 Selection of critical use(s) and justification

The critical GAPs used for the exposure assessment of the plant protection product are shown in Table 0-4. A list of all intended uses within the central zone is given in Part B, Section 0.

Justification

The critical GAP used for the exposure assessment is selected based on the highest application rate (worst-case).

6.6.2 Operator exposure (KCP 7.2.1)

6.6.2.1 Estimation of operator exposure

A summary of the exposure models used for estimation of operator exposure to the active substances during application of GLOB1310aH/Glosset Ace according to the critical use(s) is presented in Table 0-2. The outcome of the longer term exposure estimation is presented in Table 0-3. No acute exposure calculations are necessary. Detailed calculations are in 0.

The uses 7-12 of GLOB1310aH (2.0L formulation/ha) are used here as worst case and considered to cover the uses 1-6 in winter cereals (1.5L formulation/ha).

Table 0-2: Exposure models for intended uses

Critical use(s)	Winter cereals (max. 2.0 L product/ha, 1 application per season)
Model(s)	Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products; EFSA Journal

	2014;12(10):3874 calculator version: 30/03/2015

Table 0-3: Estimated operator exposure (longer term exposure)

		Aclonifen		Flufenacet	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg/day)**	**% of systemic AOEL**
Tractor mounted boom spray application (downwards) outdoors to low crops					
Application rate		1.080 kg a.s./ha (covers 0.810 kg/ha)		0.120 kg a.s./ha (covers 0.09 kg/ha)	
Spray application (AOEM**;** 75th percentile) Body weight: 60 kg	Without RPE/PPE potential exposure	0.0059670	8.52%	0.0655295	385.47%
	Work wear (arms, body and legs covered) M/L and A	0.0040263	5.75%	0.0107415	239.66%
	Work wear (arms, body and legs covered) M/L and A + gloves during mixing/loading	0.0032792	4.68%	0.008773	51.59%

zRMS conclusion
According to AOEM model calculations, it can be concluded that the exposure to aclonifen and flufenacet of operator wearing work wear (during mixing, loading and application) and gloves during mixing and loading, using GLOB1310aH on cereals at maximum application rate of 2L product/ha is acceptable. The acceptable operator exposure level (sum of % of AOELs for aclonifen and flufenacet < 100%) will not be exceeded under conditions of intended uses and consideration of the above mentioned personal protective equipment (PPE – gloves during M/L). Taking into account CLP classiffication of the formulation (Skin Sens. 1 and Carc. 2) GLOB1310aH/Glosset Ace and in-use dilutions (Skin Sens. 1) the operator should wear protective clothing, protective gloves and face/eye protection during mixing, loading and application.

6.6.2.2 Measurement of operator exposure

Since the operator exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended uses and consideration of the above mentioned personal protective equipment (PPE), a study to provide measurements of operator exposure was not necessary and was therefore not performed.

6.6.3 Worker exposure (KCP 7.2.3)

6.6.3.1 Estimation of worker exposure

Table 0-4 shows the exposure model used for estimation of worker exposure after entry into a previously treated area or handling a crop treated with GLOB1310aH according to the critical uses. Outcome of the estimation is presented in **Błąd! Nie można odnaleźć źródła odwołania.**Table 6.6-5 (longer term

exposure). Detailed calculations are in 0.

The uses 7-12 of GLOB1310aH (2.0L formulation/ha) are used here as worst case and considered to cover the uses 1-2 in winter cereals (1.5L formulation/ha).

Table 0-4: Exposure models for intended uses

Critical use(s)	Cereals (max. 1 x 2 L product/ha)
Model	Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products; EFSA Journal 2014;12(10):3874 calculator version: 30/03/2015

Table 0-5: Estimated worker exposure (longer term exposure)

		Aclonifen		Flufenacet	
Model data	**Level of PPE**	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Inspection, irrigation/Outdoor Work rate: 2 hours/day, DT_{50}: 30 days DFR: 3 µg/cm²/kg a.s./ha Interval between treatments: 365 days					
Number of applications and application rate		1 x 1.08 kg a.s./ha		1 x 0.12 kg a.s./ha	
Body weight: 60 kg	Potential TC: 12500 cm²/person/h	0.0283500	40.50%	0.0750000	441.18%
	Work wear (arms, body and legs covered) TC: 1400 cm²/person/h	0.0031752	4.54%	0.0084000	49.41%

zRMS conclusion
It is concluded that there is no unacceptable risk anticipated for the worker wearing adequate work clothing (but no PPE), when re-entering crops (for 2h/d) treated with GLOB1310aH under conditions of intended GAP uses. The acceptable worker exposure level (sum of % of AOELs for aclonifen and flufenacet < 100%) will not be exceeded under conditions of intended uses of the product. The product is applied before the emergence of seedlings (BBCH 00-09 – germination stage of crop). Due to the absence of foliage, exposure of workers towards dislodgeable foliar residues is not considered relevant for this scenario. ~~As a standard rule, it could be mentioned on the label that treated crops should not be re-entered before spray deposits on leaf surfaces have completely dried.~~ Thus, there is no need to re-enter the cereals shortly after application, only crop inspection is considered.

6.6.3.2 Refinement of generic DFR value (KCP 7.2)

Refinement of the generic Dislodgeable Foliar Residues (DFR) was not necessary since there is no risk for worker exposure.

6.6.3.3 Measurement of worker exposure

Since the worker exposure estimations carried out indicated that the acceptable operator exposure level (AOEL) will not be exceeded under conditions of intended uses and considering above mention PPE, a study to provide measurements of worker exposure was not necessary and was therefore not performed.

6.6.4 Resident and bystander exposure (KCP 7.2.2)

6.6.4.1 Estimation of resident and bystander exposure

The acute exposure assessment for bystanders covers the exposure that a resident could reasonably be expected to incur in a single day. Therefore, there is no need for a separate acute risk assessment for residents.

No bystander risk assessment is required for PPPs that do not have significant acute toxicity or the potential to exert toxic effects after a single exposure. Exposure in this case will be determined by average exposure over a longer duration, and higher exposures on one day will tend to be offset by lower exposures on other days. Therefore, exposure assessment for residents also covers bystander exposure.

Table 0-6 shows the exposure model used for estimation of resident and bystander exposure to aclonifen and flufenacet. The outcome of the estimation is presented in Table 6.6-7. Detailed calculations are in 0.

The uses 7-12 of GLOB1310aH (2.0L formulation/ha, 200/L water/ha) are used here as worst case and considered to cover the uses 1-6 in winter cereals (1.5L formulation/ha).
zRMS: Since worst case of intended uses (2.0L formulation/ha, 200/L water/ha, Table 6.6-7) covers all applications, the estimations of resident exposure provided by the applicant in tables 6.6-8 – 6.6-10 are not needed.

Table 0-6: Exposure models for intended uses

Critical use(s)	Cereals (max. 1 x 2 L product/ha)
Model	Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products; EFSA Journal 2014;12(10):3874 calculator version: 30/03/2015

Table 0-7: Estimated resident and bystander exposure (longer term exposure) (2L formulation/ha, 200L water/ha)

		Aclonifen		Flufenacet	
Model data		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Tractor mounted boom spray downwards application outdoors to low crops Buffer zone: 2-3(m) Drift reduction technology: no DT_{50}: 30 days DFR: 3 µg/cm^2/kg a.s./ha Interval between treatments: 365 days **200 L water/ha**					
Number of applications and application rate		1 x 1.080 kg a.s./ha		1 x 0.120 kg a.s./ha	
Resident child	Drift (75th perc.)	0.0031595	4.51%	0.00805740	47.40%

Body weight: 10 kg	Vapour (75th perc.)	0.0010700	1.53%	0.0010700	6.29%
	Deposits (75th perc.)	0.0012072	1.72%	0.0009710	5.71%
	Re-entry (75th perc.)	0.0038273	5.47%	0.0101250	59.56%
	Sum (mean)	0.0067710	9.67%	0.0142921	84.07%
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0007374	1.05%	0.0019280	11.34%
	Vapour (75th perc.)	0.002300	0.33%	0.0002300	1.35%
	Deposits (75th perc.)	0.0001545	0.22%	0.0004088	2.40%
	Re-entry (75th perc.)	0.0021263	3.04%	0.0056250	33.09%
	Sum (mean)	**0.0023924**	**3.42%**	**0.0059302**	**34.88%**

Table 0-8: Estimated resident exposure (longer term exposure) (2L formulation/ha, 300L water/ha)

		Aclonifen		**Flufenacet**	
Model data		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Tractor mounted boom spray downwards application outdoors to low crops Buffer zone: 2-3(m) Drift reduction technology: no DT_{50}: 30 days DFR: 3 µg/cm²/kg a.s./ha Interval between treatments: 365 days **300 L water/ha**					
Number of applications and application rate		1 x 1.080 kg a.s./ha		1 x 0.120 kg a.s./ha	
Resident child Body weight: 10 kg	Drift (75th perc.)	0.0021063	3.01%	0.0053716	31.60%
	Vapour (75th perc.)	0.0010700	1.53%	0.0010700	6.29%
	Deposits (75th perc.)	0.0012072	~~1.47%~~ 1.72%	0.0009710	5.71%
	Re-entry (75th perc.)	0.0038273	5.47%	0.0101250	59.56%
	Sum (mean)	0.0061825	~~8.65%~~ 8.83%	0.0128127	75.37%
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0004916	0.70%	0.0012853	7.56%
	Vapour (75th perc.)	0.0002300	0.33%	0.0002300	1.35%
	Deposits (75th perc.)	0.0001545	0.22%	0.0004088	2.40%
	Re-entry (75th perc.)	0.0021263	3.04%	0.0056250	33.09%
	Sum (mean)	0.0022745	3.25%	0.0056249	33.09%

Table 0-9: Estimated resident exposure (longer term exposure) (1.5L formulation/ha, 150L water/ha)

		Aclonifen		Flufenacet	
Model data		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Tractor mounted boom spray downwards application outdoors to low crops Buffer zone: 2-3(m) Drift reduction technology: no DT_{50}: 30 days DFR: 3 µg/cm²/kg a.s./ha Interval between treatments: 365 days **150 L water/ha**					
Number of applications and application rate		1 x 0.810 kg a.s./ha		1 x 0.090 kg a.s./ha	
Resident child Body weight: 10 kg	Drift (75th perc.)	0.0031595	4.51%	0.0040287	23.70%
	Vapour (75th perc.)	0.0010700	1.53%	0.0010700	6.29%
	Deposits (75th perc.)	0.0007738	~~1.11%~~ 1.29%	0.0003641	2.14%
	Re-entry (75th perc.)	0.0028704	4.10%	0.0075938	44.67%
	Sum (mean)	0.0056908	~~8.13%~~ 8.27%	0.0096105	56.63%
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0007374	1.05%	0.0009640	5.67%
	Vapour (75th perc.)	0.0002300	0.33%	0.0002300	1.35%
	Deposits (75th perc.)	0.0001159	0.17%	0.0001533	0.90%
	Re-entry (75th perc.)	0.0015947	2.28%	0.0042188	24.82%
	Sum (mean)	0.0019403	2.77%	0.0041640	24.49%

Table 0-10: Estimated resident exposure (longer term exposure) (1.5L formulation/ha, 300L water/ha)

		Aclonifen		Flufenacet	
Model data		**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**	**Total absorbed dose (mg/kg bw/day)**	**% of systemic AOEL**
Tractor mounted boom spray downwards application outdoors to low crops Buffer zone: 2-3(m) Drift reduction technology: no DT_{50}: 30 days DFR: 3 µg/cm²/kg a.s./ha Interval between treatments: 365 days 300 L water/ha					
Number of applications and application rate		1 x 0.810 kg a.s./ha		1 x 0.090 kg a.s./ha	
Resident child Body weight: 10 kg	Drift (75th perc.)	0.0015798	2.26%	0.0040287	23.70%
	Vapour (75th perc.)	0.0010700	1.53%	0.0010700	6.29%
	Deposits (75th perc.)	0.0009054	1.29%	0.0007283	4.28%

	Re-entry (75th perc.)	0.0028704	4.10%	0.0075938	44.67%
	Sum (mean)	0.0049044	7.01%	0.0098771	58.10%
Resident adult Body weight: 60 kg	Drift (75th perc.)	0.0003687	0.53%	0.0009640	5.67%
	Vapour (75th perc.)	0.0002300	0.33%	0.0002300	1.35%
	Deposits (75th perc.)	0.0001159	0.17%	0.0003066	1.80%
	Re-entry (75th perc.)	0.0015947	2.28%	0.0042188	24.82%
	Sum (mean)	0.0017633	2.52%	0.0042762	25.15%

zRMS conclusion
It is concluded that there is no unacceptable risk anticipated for the resident after long-term exposure or bystander after accidental short-term exposure, wearing light clothing, in crops treated with GLOB1310aH under conditions of intended GAP uses. The acceptable resident/bystander (child and adult) exposure level (sum of % of AOELs for aclonifen and flufenacet < 100%) will not be exceeded under conditions of intended uses of the product.
No bystander acute exposure estimation is required since no acute acceptable operator exposure values (AAOELs) have been set for aclonifen and flufenacet, the active substances of a product GLOB1310aH.
However taking into account harmonised classification (CLP) of a.s. aclonifen as Skin Sens. 1A (GCL≥0.1%), the spray dilution is considered as sensitising based on requirements of Regulation (EC) No. 1272/2008. During MS consultations further risk mitigation measures are proposed to protect residents and bystanders, who cannot protect themselves with PPE from the sensitising properties of the spray drift. Therefore at least 50% drift reduction for protection residents and bystanders is recommended on the label.

6.6.4.2 Measurement of resident and/or bystander exposure

Since the resident and/or bystander exposure estimations carried out indicated that the acceptable resident and bystander exposure level (AOEL) for aclonifen and flufenacet will not be exceeded under conditions of intended uses and considering above mentioned risk mitigation measures for the highest intended dose of 2.0L formulation/ha (drift reduction technology of 50%) and no mitigation measures for the dose of 1.5L formulation/ha, a study to provide measurements of resident/bystander exposure was not necessary and was therefore not performed.

6.6.5 Combined exposure

The product is a mixture of two active substances.

6.6.5.1 Exposure assessment of Aclonifen and Flufenacet in Glosset Ace/GLOB1310aH

The product is a mixture of two active substances. From a scientific point of view it is regarded necessary to take into account potential combination effects. However, the evaluation of cumulative or synergistic effects as requested by Art. 4 (3b) of Regulation (EC) No. 1107/2009 should only be performed when harmonised “scientific methods accepted by the Authority to assess such effects are available.”

Currently no EU-harmonised guidance is available on the risk assessment of combined exposure to multiple active substances. Most assessment approaches employed up to now make use of the Hazard Index (HI) concept. It is therefore suggested to use this as a first-tier assessment.

Combined exposure is calculated as the sum of the component exposures without regard to the mode of

action or mechanism/target of toxicity. Initially, the individual Hazard Quotients (HQ) are calculated for all active substances in the PPP by assessing the exposure according to appropriate models and dividing the individual exposure levels by the respective systemic AOEL. This is equivalent to the predicted exposure as % of systemic AOEL converted to decimal. The Hazard Index (HI) is the sum of the individual HQs.

Table 0-11: Risk assessment from combined exposure

Application scenario		Aclonifen	Flufenacet	Estimated exposure / AAOEL (HQ)
Operators –vehicle mounted downwards application to low crops. Work wear (arms, body and legs covered) M/L (Max. intended application rate of 2.0L formulation/ha)		0.0575	2.39	**2.4475**
Operators –vehicle mounted downwards application to low crops. Work wear (arms, body and legs covered) M/L and A + **PPE** - gloves during mixing/loading (Max. intended application rate of 2.0L formulation/ha)		0.0468	0.5159	0.5627
Worker (Max. intended application rate of 2.0L formulation/ha)	Potential	0.405	4.4118	4.8168
	work wear	0.0454	0.4941	0.5395
Resident (covering Bystander) – child 2L formulation/ha, 200L water/ha, drift reduction	Drift	0.0602	0.4740	0.5342
	Vapour	0.0153	0.0629	0.0782
	Deposits	0.0172	0.0571	0.0743
	Re-entry	0.0547	0.5956	0.6503
	Mean of all pathways	0.1051	0.8407	0.9458
Resident (covering Bystander) – adult 2L formulation/ha, 200L water/ha, drift reduction	Drift	0.0140	0.1134	0.1274
	Vapour	0.0033	0.0135	0.0168
	Deposits	0.0022	0.0240	0.0460
	Re-entry	0.0304	0.3309	0.3613
	Mean of all pathways	0.0359	0.3488	0.3847
Resident (covering Bystander) – child 2L formulation/ha, 300L water/ha	Drift	0.0301	0.316	0.3461
	Vapour	0.0153	0.0629	0.0782
	Deposits	~~0.0147~~ 0.0172	0.0571	~~0.0718~~ 0.0743
	Re-entry	0.0547	0.5956	0.6503
	Mean of all pathways	~~0.0865~~ 0.0883	0.7537	~~0.8402~~ 0.8420
Resident (covering Bystander) – adult 2L formulation/ha, 300L water/ha	Drift	0.007	0.0756	0.0826
	Vapour	0.0033	0.0135	0.0168
	Deposits	0.0022	0.024	0.0262
	Re-entry	0.0304	0.3309	0.3613
	Mean of all pathways	0.0325	0.3309	0.3634
Resident (covering Bystander) – child 1.5L formulation/ha, 150L water/ha	Drift	0.0451	0.2370	0.2821
	Vapour	0.0153	0.0629	0.0782
	Deposits	~~0.0111~~ 0.0129	0.0214	~~0.0325~~ 0.0343
	Re-entry	0.0410	0.4467	0.4877
	Mean of all pathways	~~0.0813~~ 0.0827	0.5653	~~0.6466~~ 0.6480
	Drift	0.0105	0.0567	0.0672

Application scenario		Aclonifen	Flufenacet	Estimated exposure / AAOEL (HQ)
Resident (covering By-stander) – adult 1.5L formulation/ha, 150L water/ha	Vapour	0.0033	0.0135	0.0168
	Deposits	0.0017	0.0090	0.0107
	Re-entry	0.0228	0.2482	0.2710
	Mean of all pathways	0.0277	0.2449	0.2726
Resident (covering By-stander) – child 1.5L formulation/ha, 300L water/ha	Drift	0.0226	0.2370	0.2596
	Vapour	0.0153	0.0629	0.0782
	Deposits	0.0129	0.0428	0.0557
	Re-entry	0.0410	0.4467	0.4877
	Mean of all pathways	0.0701	0.5810	0.6511
Resident (covering By-stander) – adult 1.5L formulation/ha, 300L water/ha	Drift	0.0053	0.0567	0.0620
	Vapour	0.0033	0.0135	0.0168
	Deposits	0.0017	0.0180	0.0197
	Re-entry	0.0228	0.2482	0.2710
	Mean of all pathways	0.0252	0.2515	0.2767

The Hazard Index is < 1. Thus, combined exposure to all active substances in GLOB1310aH/Glosset Ace is not expected to present a risk for operators and workers, thus no further refinement of the assessment is required.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCA 5.8.1			In vitro mammalian chromosome aberration in human lymphocytes of Flufenacet metabolite M2. LEMI Laboratory, Study No.: 2015-FRU-4, GLP Unpublished	N	Task Force Flufenacet
KCA 5.8.1			In vitro Mammalian Cell Gene Mutation Test with Flufenacet Sulfonic acid Na salt in Hprt gene using V79 cell line Vanta Bioscience Limited Study Number: 19-046-G GLP Unpublished	N	Globachem NV
KCA 5.8.1	Savineau, C.	2016a	Bacterial reverse mutation test of Flufenacet Metabolite M1 in mutated "*Salmonella typhimurium his*-". LEMI Laboratory, Study No.: 2015-FRU-1 GLP Unpublished	N	Task Force Flufenacet
KCA 5.8.1			In vitro mammalian cell gene mutation test with flufenacet metabolite M1. LEMI Laboratory,	N	Task Force Flufenacet

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
			Study No.: 2015-FRU-5, GLP Unpublished		
KCA 5.8.1			In vitro chromosome aberration in human lymphocytes of Flufenacet metabolite M1. LEMI Laboratory, Study No.: 2015-FRU-3, GLP Unpublished	N	Task Force Flufenacet
KCP 7.3			Aclonifen – In vitro percutaneous penetration of [^{14}C]Aclonifen formulated as GLOB1310aH through Human Skin Membranes, IES, Swtizerland Study No. 20200082 Innovative Environmental Services (IES) Ltd, Switzerland GLP Unpublished	N	Globachem NV

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

Appendix 2 Detailed evaluation of the studies relied upon

A 2.1 Statement on bridging possibilities

A 2.2 Acute oral toxicity (KCP 7.1.1)

No new studies on acute oral toxicity are submitted within this dossier.

A 2.3 Acute percutaneous (dermal) toxicity (KCP 7.1.2)

No new studies on acute dermal toxicity are submitted within this dossier.

A 2.4 Acute inhalation toxicity (KCP 7.1.3)

No new studies on acute inhalation toxicity are submitted within this dossier. Please refer to Part C.

A 2.5 Skin irritation (KCP 7.1.4)

No new studies on skin irritation properties are submitted within this dossier.

A 2.6 Eye irritation (KCP 7.1.5)

No new studies on eye irritation properties are submitted within this dossier.

A 2.7 Skin sensitisation (KCP 7.1.6)

No new studies on skin sensitisation properties are submitted within this dossier.

A 2.8 Supplementary studies for combinations of plant protection products (KCP 7.1.7)

No new studies are submitted within this dossier.

A 2.9 Data on co-formulants (KCP 7.4)

A 2.9.1 Material safety data sheet for each co-formulant

Information regarding material safety data sheets of the co-formulants can be found in the confidential dossier of this submission (Registration Report - Part C).

A 2.9.2 Available toxicological data for each co-formulant

Available toxicological data for each co-formulant can be found in the confidential dossier of this submission (Registration Report - Part C).

A 2.10 Studies on dermal absorption (KCP 7.3)

A 2.10.1 Study 1 – Aclonifen in GLOB1310aH

Comments of zRMS:	The study conducted according to relevant OECD guidelines and in GLP principles is considered acceptable. The dermal absorption of aclonifen formulated as product GLOB1310aH through human dermatomed skin was determined *in vitro*. The amount of applied dose penetrating within 24 hours was determined to be 0.02 (± 0.02)% and 1.58 (± 0.65)% for the formulation concentrate and the 1:200 spray dilution, respectively. The dermal penetration estimates to be used for risk assessment were set at 0.043% and 2.10% for the formulation concentrate and the 1:200 spray dilution (lowest concentration used) based on the EFSA guidance criteria (EFSA Journal 2017;15(6):4873). These values are based on the potentially absorbed dose defined as the amount in the receptor fluid, the receptor chamber wash, the skin and stratum corneum (except for the first 2 tape strips). The normalized values were used and the standard deviation was added to the mean value since the standard deviation was more than 25% of the mean value

Reference	KCP 7.3
Report	Aclonifen – In vitro percutaneous penetration of [^{14}C]Aclonifen formulated as GLOB1310aH through Human Skin Membranes, Report No. 20200082
Guideline(s)	Yes, OECD 428
Deviations	No
GLP	Yes
Acceptability	Yes

Materials and methods

Test material	Name (Lot/Batch No.)	Aclonifen
	Test preparation	Spiking
	Radiochemical purity	99.2%
Product	Name (Lot/Batch No.)	GLOB1310aH
	Company code	GLOB1310aH
	Concentration a.s.	540 g/L
	Type of formulation	SC
Blank product	Name (Lot/Batch No.)	GLOB1310aH Blank formulation (GLO-20F-2306A-Blank B)
	Concentration a.s.	Na

Test system		
Diffusion cell	Type of diffusion cell	Flow-through
	(If dynamic) Flow rate	3 mL/h

	Exposed skin area	1 cm^2
	Cover	Unoccluded
Membrane	Skin type	Dermatomed
	Skin thickness range	400 µm
	Skin donor age	25-43 years
	Skin donor sex	Male and female
	Site	Abdomen
	Source	Surgery
	Integrity test	Yes (permeability coefficient)
Receptor	Receptor medium	phosphate physiological buffered saline with 5% (w/v) of Volpo N20
	Solubility in receptor medium	1826 µg/mL
Sample time	Exposure time	6 hours
	Sampling duration	24 hours
Sampling	Sample intervals	1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 hours
Washing	Skin wash	Post-exposure (3 times / 0.5 mL with mild shower gel solution 1% in water)
Final procedure	Tape stripping	Yes
	TS 1-2 analysed separately?	Yes
Remarks	None	

Tested doses	Concentrate	Dilution 1 (1:200)
Target concentration [mg/mL]	523.6	2.68
Surface area dose [µg/cm^2]	5236	26.8
Total dose [µg/cell]	5236.289	26.8163194
Specific activity [kBq/mL]	2134	906
No. of donors	5	5
No. of replicates used/valid replicates*	9/10	9/9

* Justification for excluded cells: For one cell of the high dose level, i.e. Cell 16, the total recovery amounted for < 90% of the dose, therefore these values were excluded from mean calculation: for one cell (Cell 16) the high dose level the total recovery amounted for <90%, these values were excluded from the calculations

Results and discussions

Table A 1: In-vitro dermal penetration of active substance Aclonifen formulated as product GLOB1310aH through human skin - Recovery data

Dose group	High dose (Formulation concentrate)		Low dose (Spray dilution 1:200)	
Target concentration [mg/mL]	523.6		2.68	
Target dose [µg/cm^2]	5236		26.8	
Mean actual applied dose [µg/cm^2]	5236.289		28.816	
	Recovery [%]		Recovery [%]	
	Mean	S.D.	Mean	S.D.
Dislodgeable dose				
Skin washing (sum of all wasings)	99.23	4.61	99.42	2.53
Donor chamber wash	0.02	0.02	0.17	0.16
Dose associated to skin				
Tape strips: 1st sample, strips 1 + 2	0.08	0.14	1.13	0.53
Tape strips: 2nd sample; strips 3 - n	0.00	0.00	0.02	0.01
Skin preparation	0.00	0.00	0.11	0.03

Absorbed dose				
Receptor fluid	0.02	0.02	1.45	0.64
Receptor chamber wash	0.00	N/A	0.02	N/A
Total recovery[1]				
Absorption essentially complete at end of study (>75% absorption within half the study duration) [%Absorption at $t_{0.5}$]	No [15.37% ±17.22]		No [35.32% ±3.03]	
If no: Absorption estimates = absorbed dose + skin preparation + tape strips sample 2)[2]	0.0257	0.02206	1.6033	0.6481
If yes: Absorption estimates = absorbed dose + skin preparation	-	-	-	-
Absorption estimate normalised[3]	0.0257		1.6033	
Relevant absorption estimate[4]	0.043		2.1024	
Absorption estimates used for risk assessment[5]	**0.043**		**2.10**	

[1] Values may not calculate exactly due to rounding of figures

[2] In accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2017;15(6):4873) the radioactivity in the second tape-strip pool (3rd to nth tape strip) is considered potentially absorbable if less than 75% of the absorption occurred in the first half of the study (see Table 7.6.2-1) Finally, the skin preparation is also considered potentially absorbable.

[3] According to the EFSA Guidance on Dermal Absorption, cells with insufficient recovery (< 95%) can be corrected by normalisation of absorption estimate to 100% recovery; explanation should be included.

[4.] In accordance with the EFSA Guidance on Dermal Absorption, one standard deviation was added to the mean% dermal penetration in cases where the standard deviation was ≥ 25% of the mean value.

[5] Relevant absorption estimate was rounded to the required number of significant figures.

N/A: not applicable

Remarks

Justification for excluded cells: For one cell of the high dose level, i.e. Cell 16, the total recovery amounted for < 90% of the dose, therefore these values were excluded from mean calculation: for one cell (Cell 16) the high dose level the total recovery amounted for <90%, these values were excluded from the calculations

Conclusion/endpoint:

The dermal penetration of aclonifen formulated as GLOB1310aH through human dermatomed skin was determined in vitro. The dermal penetration estimates to be used for risk assessment were set at 0.043 % for the concentrate, and 2.10 % for the dilution based on the EFSA guidance criteria and the calculations in the Excel BfR sheet.

A 2.11 Other/Special Studies

Comments of zRMS:	The study conducted according to relevant OECD guidelines and in GLP principles is considered acceptable. Flufenacet metabolite M2 is considered to be non clastogenic for mammalian cells with and without metabolic activation in vitro.

Reference	KCA 5.8.1
Report	In vitro mammalian chromosome aberration in human lymphocytes of Flufenacet metabolite M2. LEMI Laboratory, , Study No.: 2015-FRU-4.
Guideline(s)	Yes, OECD 473
Deviations	No
GLP	Yes
Acceptability	Yes

Materials and Methods

The assay was performed in both the absence and the presence of an appropriate metabolic activation system (Rat liver microsome fraction) to detect promutagenes agents.

Human lymphocytes culture stimulated by phytohemagglutinin A are exposed to the test item (two original solutions and dilutions) for 4 hours and 20 hours in the absence of metabolic activation and for 3 hours in the presence of metabolic activation. The cultures were treated to block cells in the metaphase. Two hours and a half later cells are harvested and stained with Giemsa. The metaphases are analysed microscopically (x1000) for identifying and counting chromosomal aberrations, polyploidies and endoreduplications.

Test item: flufenacet M2, purity 99.3%, tested concentrations 2000-800-320-128 µg/mL
Absolute negative control: Ham F12
Negative control (solvent): DMSO (0.67%)
Positive controls: without metabolic activation: Mitomycin C (0.25 µg/mL)
With metabolic activation: Cyclophosphamide monohydrate (50 µg/mL)

Assay conditions:

- Cell culture: lymphocytes are stimulated 48h in the presence of 2% (v/v° phytohemagglutinin A (PHA). Cell cultures are incubated at 37°C in a humid atmosphere containing 5% (v/v) CO2.

Results

Mitotic Index without Metabolic activation (-S9mix)

Series	Assay	Concentration	Cells observed	Cells in metaphase	Mitotic Index	Mitotix Index Reduction (%)
Negative control (culture medium)	1	-	1000	121	12.10	-
Positive control (Mitomycin C)		0.25 µg/mL	500	51	10.20	15.7
Solvent control (DMSO)		-	1000	101	10.10	-
Flufenacet metabolite M2		128 µg/mL	1058	116	10.96	-
		320 µg/mL	1000	106	10.60	-
		800 µg/mL	1000	98	9.80	3.0
		2000 µg/mL	1008	81	8.04	20.4

Negative control (culture medium)	2	-	1004	130	12.95	-
Positive control (Mitomycin C)		0.25 µg/mL	503	36	7.16	44.7
Solvent control (DMSO)		-	1000	131	13.10	-
Flufenacet metabolite M2		128 µg/mL	1005	123	12.24	6.6
		320 µg/mL	1003	107	10.67	18.5
		800 µg/mL	1000	72	7.20	45.0
		2000 µg/mL	1000	52	5.20	60.3

Assay 1: 4 hours without metabolic activation; Assay 2: 20 hours without metabolic activation

Mitotic Index with Metabolic activation (+S9mix 10% (v/v))

Series	Assay	Concentration	Cells observed	Cells in metaphase	Mitotic Index	Mitotix Index Reduction (%)
Negative control (culture medium)	1	-	1000	88	8.80	-
Positive control (Cyclophosphamide)		50 µg/mL	500	24	4.80	45.5
Solvent control (DMSO)		-	1000	88	8.80	-
Flufenacet metabolite M2		128 µg/mL	1000	86	8.60	2.3
		320 µg/mL	1000	81	8.10	8.0
		800 µg/mL	1000	73	7.30	17.0
		2000 µg/mL	1000	83	8.30	5.7

Assay 1: 3 hours with metabolic activation S9 10% v/v

Chromosome aberrations in human lymphocytes in the absence of metabolic activation

Series	Assay	Concentration (µg/mL)	Cells observed	Aberrations (type and number)													
				Chromosomal						Chromatide						Other	
				G***	C	M	D	CR	R	g***	c	ic	tr	qr	d	CP	cp
Negative control (culture medium)	1	-	300	0	1	0	0	0	0	4	1	0	0	0	0	0	0
Positive control (Mitomycin C)		0.25	26	0	0	0	0	0	0	0	2	0	1	3	0	0	0
Solvent control (DMSO)		-	302	1	1	0	0	0	0	3	2	0	0	0	0	0	0
Flufenacet metabolite M2		320	300	1	0	0	0	0	0	3	3	0	0	0	0	0	0
		800	300	0	1	0	0	0	0	2	0	0	0	0	0	0	0
		2000	300	0	1	0	0	0	0	3	3	0	0	0	0	0	0
Negative control (culture medium)	2	-	301	0	1	0	0	0	0	3	2	0	0	0	0	0	0
Positive control (Mitomycin C)		0.25	25	0	2	0	0	0	0	2	8	0	2	1	0	0	0
Solvent control (DMSO)		-	301	0	1	0	0	0	0	7	2	0	0	0	0	0	0

Flufenacet metabolite M2		128	300	1	0	0	0	0	0	4	3	0	0	0	0	0	0
		320	300	4	3	0	0	0	0	5	4	0	0	0	0	0	0
		800	300	1	1	0	0	0	0	6	7	0	1	0	0	0	0

Series	**Assay**	**Concentration**	**Cells observed**	**Total aberration**	**Aberration/cell**	**Cells with aberration**	**Cells with aberration (%)**	**X^2**	**p**
Negative control (culture medium)	1	-	300	2	0.007	2	0.67	-	-
Positive control (Mitomycin C)		0.25 µg/mL	26	6	0.231	6	23.08	50.20	<0.001
Solvent control (DMSO)		-	3002	3	0.010	3	0.99	-	-
Flufenacet metabolite M2		320 µg/mL	300	3	0.010	3	1.00	0.00	NS
		800 µg/mL	300	1	0.003	1	0.33	0.99	NS
		2000 µg/mL	300	4	0.013	4	1.33	0.15	NS
Negative control (culture medium)	2	-	300	3	1.010	3	1.00	-	-
Positive control (Mitomycin C)		0.25 µg/mL	25	13	0.520	12	48.00	115.79	<0.001
Solvent control (DMSO)		-	301	3	0.010	3	1.00	-	-
Flufenacet metabolite M2		128 µg/mL	300	3	0.010	9	3.00	3.10	NS
		320 µg/mL	300	7	0.023	7	2.33	1.65	NS
		800 µg/mL	300	9	0.030	9	3.00	3.10	NS

NS: not statistically significant, p≥0.05. Aberration of chromosome type: Gap or achromatic lesion (G**), Break or terminal deletion (C), Dicentric chromosome exchange (D or DF), comples rearrangement exchange (CR), ring exchange (R or RF), Minutes (M). Aberrations of chromatidic type: gap (g**), break ((c), meadian deletion (d), chromosome interchange (ci), triradial exchange (tr), quadriradial exchange (qr). Other events: pulverises chromosome (PC), pulverised cell (pc).

Chromosome aberrations in human lymphocytes in the presence of metabolic activation

Series	**Assay**	**Concentration (µg/mL)**	**Cells observed**	**Aberrations (type and number)**													
				Chromosomal						**Chromatide**						**Other**	
				G***	**C**	**M**	**D**	**CR**	**R**	**g*****	**c**	**ic**	**tr**	**qr**	**d**	**CP**	**cp**
Negative control (culture medium)	1	-	300	0	1	0	0	0	0	4	2	0	0	0	0	0	0
Positive control (Cyclophosphamide)		50	25	1	2	0	0	0	0	2	2	0	0	0	0	0	0
Solvent control (DMSO)		-	300	2	0	0	0	0	0	2	3	0	0	0	1	0	0
Flufenacet metabolite M2		320	300	0	1	0	0	0	0	3	1	0	0	0	0	0	0
		800	300	0	1	0	0	0	0	1	2	0	0	0	0	0	0
		2000	300	0	0	0	0	0	0	1	3	0	0	0	0	0	0

Series	**Assay**	**Concentration**	**Cells observed**	**Total aberration**	**Aberration/cell**	**Cells with aberration**	**Cells with aberration (%)**	**X^2**	**p**
Negative control (culture medium)	1	-	300	3	0.010	3	1.00	-	-
Positive control		50 µg/mL	25	4	0.160	4	16.00	24.64	<0.001

(Cyclo-phospha-mide)									
Solvent control (DMSO)		-	300	4	0.013	4	1.33	-	-
Flufenacet metabolite M2		320 µg/mL	300	2	0.007	2	0.67	0.67	NS
		800 µg/mL	300	3	0.010	3	1.00	0.14	NS
		2000 µg/mL	300	3	0.010	3	1.00	0.14	NS

NS: not statistically significant, p≥0.05. Aberration of chromosome type: Gap or achromatic lesion (G**), Break or terminal deletion (C), Dicentric chromosome exchange (D or DF), comples rearrangement exchange (CR), ring exchange (R or RF), Minutes (M). Aberrations of chromatidic type: gap (g**), break ((c), meadian deletion (d), chromosome interchange (ci), triradial exchange (tr), quadriradial exchange (qr). Other events: pulverises chromosome (PC), pulverised cell (pc).

Conclusions

Flufenacet metabolite M2 is not considered clastogenic in the test system used (human lymphocytes) in the conditions of the assay.

Comments of zRMS:	The study conducted according to relevant OECD guidelines and in GLP principles and is acceptable. The test material is considered non mutagenic in in vitro mammalian cell gene mutation test both with and without metabolic activation.

Reference	KCA 5.8.1
Report	In vitro mammalian Cell Gene Mutation Test with Flufenacet Sulfonic acid Na salt in Hprt gene using V79 cell line. 2019. Study No.: 19-046-G
Guideline(s)	Yes, OECD 476
Deviations	No
GLP	Yes
Acceptability	Yes

Materials and Methods

The test item, Flufenacet Sulfonic acid Na salt was evaluated to assess its ability to induce gene mutations in reporter genes specifically the endogenous hypoxanthine-guanine phosphoribosyl transferase gene (Hprt) in the presence and absence of an exogenous mammalian metabolic activation system (S9) using V79 cells.

The test item was found soluble in Type I Milli Q water at 100 mg/mL. Hence Type I Milli Q water was selected as a vehicle to prepare the stock and dilutions of the test item. Precipitation and pH of the Test Item in the culture medium was tested before cytotoxicity test. No precipitation of test item in the culture medium was observed at 2000 µg/mL. The observed pH was 7.4 at 0 and 4 hours of incubation at 37±1 °C.

On the basis of test item solubility, precipitation and pH the initial cytotoxicity was performed at the test concentration of 125, 250, 500, 1000 and 2000 µg/mL. Two treatment conditions were maintained throughout the experiment viz., short term exposure (3 to 6 hours) in the presence of metabolic activation (1 % v/v S9 Mix), short term exposure (3 to 6 hours) in the absence of metabolic activation. The selected concentrations were treated along with vehicle control (Type I Milli Q water) and single culture was maintained for all the treatment conditions.

Cytotoxicity was evaluated by relative survival and it includes cloning efficiency (CE) of cells plated immediately after treatment adjusted by any loss of cells during treatment as compared with adjusted cloning efficiency in vehicle control.

The cells were exposed to Flufenacet Sulfonic acid Na salt at 125, 250, 500, 1000 and 2000 µg/mL.

Test item: Flufenacet sulfonic acid Na salt, purity 99.2%
Vehicle control: Type I Milli Q water
Positive control: with metabolic activation (+S9): Benzo(a)pyrene (10µg/mL)
Without metabolic activation (-S9): Ethylmethane Sulfonate (150µg/mL)
Test system: V79 cells derived from the lung of the Chinese hamster, modal chromosome number 22±2
Growth medium: Minimum essential medium
Metabolic activation system: Aroclor 1254 inducing rat liver S9 fraction was used in the experiment. The S9 fraction with cofactor, in the form of S9 mix, was prepared fresh prior to treatment and maintained on ice throughout the experiment. The S9 fraction was buffered and supplemented with the essential co-factors to form the "S9 mix".

Results

Gene mutation Assay with metabolic activation system

Treatment Code	Test Concentrations (µg/mL)	At the start of treatment X 10^6 Cells/mL	At the end of treatment X 10^6 Cells/mL	Adjusted CE	Relative Survival (%)
VC	0 (VC)	6.13	6.28	0.96	100
T4	250	6.13	5.70	0.89	92.71
T3	500	6.13	6.04	0.84	87.50
T2	1000	6.13	5.72	0.83	86.46
T1	2000	6.13	5.98	0.89	92.71
PC	10 (BoA)	6.13	5.73	0.70	72.92

Note: T- Treatment, VC- Vehicle Control, CE- Cloning Efficiency

Gene mutation Assay without metabolic activation system

Treatment Code	Test Concentrations (µg/mL)	At the start of treatment X 10^6 Cells/mL	At the end of treatment X 10^6 Cells/mL	Adjusted CE	Relative Survival (%)
VC	0	6.22	5.76	0.85	100
T4	250	6.22	5.68	0.85	100
T3	500	6.22	5.94	0.82	96.47
T2	1000	6.22	5.48	0.76	89.41
T1	2000	6.22	5.44	0.77	90.59
PC	150 (EMS)	6.22	5.92	0.64	75.29

Note : T- Treatment, VC : Vehicle Control (Type I Milli Q water), PC: Positive control, CE : Cloning Efficiency, CE = Number of colonies / Number of cells plated, BoA : Benzo(a)pyrene, EMS : Ethyl Methanesulfonate Adjusted Cloning Efficiency (ACE) : CE × No. of cells at the end of treatment/ No. of Cells at the beginning of the treatment, Relative Survival (RS) : ACE (Treated)/ ACE (Vehicle Control) x 100.

Gene Mutation assay: Summary of mutation frequency

Treatment Code	Test Concentrations (µg/mL)	Mutation Frequency for 10^6 Cells (Mean & SD)	
		With Metabolic Activation System	Without Metabolic Activation System
VC	0	6.77 ± 1.22	8.88 ± 1.30
T4	250	6.28 ± 0.85	6.43 ± 1.46
T3	500	6.95 ± 1.10	9.34 ± 2.00
T2	1000	7.43 ± 1.25	6.43 ± 1.33
T1	2000	8.68 ± 2.67	9.03 ± 2.87
PC	10 (BoA)	244.07 ± 36.24	NA
PC	150 (EMS)	NA	353.71 ± 21.43

Note: VC : Vehicle Control (Type I Milli Q water), PC: Positive control, T1 to T4 : Test Concentrations starting from higher to lower, , BoA : Benzo(a)pyrene, EMS : Ethyl Methanesulfonate, NA : Not Applicable.

Conclusions

Based on the results obtained, Flufenacet Sulfonic acid Na salt is non mutagenic and does not to induce forward mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus of V79 cells at all the tested concentrations both in presence and absence of metabolic activation under the test conditions employed.

Comments of zRMS:	The study conducted according to relevant OECD guidelines and in GLP principles and is acceptable. The test material is considered to be non-mutagenic in Salmonella typhimurium reverse mutation test both with and without metabolic activation.

Reference	KCA 5.8.1
Report	Bacterial reverse mutation test of Flufenacet Metabolite M1 in mutated “Salmonella typhimurium his-”. LEMI Laboratory. Savineau, C. 2016a. Study No.: 2015-FRU-1.
Guideline(s)	Yes, OECD 471
Deviations	No
GLP	Yes
Acceptability	Yes

Materials and methods

Five doses of flufenacet metabolite M1were tested for the capacity to induce reverse mutation in five *Salmonella typhimurium* strains. This study was performed in the absence and presence of metabolic activation. Two independent assays were carried out.

For assay No. 1 five doses (50 to 5000 µg/plate) of the M1 solution were put in contact with the strains in the absence and presence of a metabolic activation system (+S9mix 10% v/v). For assay No. 2 five doses (50 to 5000 µg/plate) of the M1 solution were put in contact with the strains in the absence of metabolic activation and with preincubation in the presence of metabolic activation system (+S9mix 10% v/v). For the two assays, negative and positive controls were carries out in parallel.

Test item: flufenacet metabolite M1, purity 99.8%
Negative control-Solvent: water, DMSO, NaCl 0.15M
Positive control: 2-Nitrofluorene, Sodium Azide, 9-Aminoacridine, 2-Anthramine, Benzo-a-pyrene, Mitomycin C
Bacterial strains: *Salmonella typhimurium* TA98, *Salmonella typhimurium* TA1537, *Salmonella typhimurium* TA100, *Salmonella typhimurium* TA 1535, *Salmonella typhimurium* TA102.

Results

Mutagenic activity -TA1535

Serie	Dose/plate	Assay 1 (-S9 mix)			Serie	Dose/plate	Assay 1 with 10% S9-mix without preincubation		
		Mean	SD	R			Mean	SD	R
Negative control	**100 µL**	10.67	1.53	-	**Negative control**	**100 µL**	16.33	4.16	-
Positive control solvent	**5 µL**	10.00	2.65	-	**Positive control solvent**	**5 µL**	12..67	2.52	-
Positive control: Sodium azide	**5µg in 5µL**	504.67	71.82	50.47	**Positive control: 2-Anthramine**	**5µg in 5µL**	138.33	31.34	10.92
Vehicle	**50 µL**	11.33	3.79	-	**Vehicle**	**50 µL**	10.67	3.06	-
Flufenacet metabolite M1	**5000 µg**	9.33	3.06	0.82	**Flufenacet metabolite M1**	**5000 µg**	12.33	3.06	1.16
	1500 µg	6.00	2.00	0.53		**1500 µg**	11.67	2.08	1.09
	500 µg	7.67	1.53	0.68		**500 µg**	11.67	1.53	1.09
	150 µg	8.67	3.79	0.76		**150 µg**	13.00	1.73	1.22
	50 µg	7.67	3.06	0.68		**50 µg**	10.33	2.52	0.97
Serie	**Dose/plate**	**Assay 2 (-S9 mix)**			**Serie**	**Dose/plate**	**Assay 2 with 10% S9-mix without preincubation**		
		Mean	**SD**	**R**			**Mean**	**SD**	**R**
Negative control	**100 µL**	12.00	2.65	-	**Negative control**	**100 µL**	12.67	1.15	-
Positive control solvent	**5 µL**	10.67	6.03	-	**Positive control solvent**	**5 µL**	11.67	4.04	-
Positive control: Sodium azide	**5µg in 5µL**	709.67	56.57	66.53	**Positive control: 2-Anthramine**	**5µg in 5µL**	45.33	5.69	3.89
Vehicle	**50 µL**	9.67	1.15	-	**Vehicle**	**50 µL**	10.67	0.58	-
Flufenacet metabolite M1	**5000 µg**	6.33	1.15	0.66	**Flufenacet metabolite M1**	**5000 µg**	11.67	3.06	1.09
	1500 µg	7.00	2.65	0.72		**1500 µg**	9.67	1.53	0.91
	500 µg	11.67	2.31	1.21		**500 µg**	8.00	3.00	0.75
	150 µg	9.33	3.51	0.97		**150 µg**	11.00	3.61	1.03
	50 µg	9.00	4.00	0.93		**50 µg**	11.67	4.04	1.09

Mutagenic activity -TA1537

Serie	Dose/plate	Assay 1 (-S9 mix)			Serie	Dose/plate	Assay 1 with 10% S9-mix without preincubation		
		Mean	SD	R			Mean	SD	R
Negative control	**100 µL**	4.33	2.08	-	**Negative control**	**100 µL**	9.00	2.00	-
Positive	**20 µL**	8.00	2.65	-	**Positive**	**20 µL**	5.67	3.06	-

control solvent					**control solvent**				
Positive control: 9-Aminoacridine	**5µg in 20µL**	1778.67	140.05	222.33	**Positive control: 2-Anthramine**	**2µg in 20µL**	36.33	9.61	6.41
Vehicle	**50 µL**	5.67	3.79	-	**Vehicle**	**50 µL**	10.33	4.04	-
Flufenacet metabolite M1	**5000 µg**	3.00	1.00	0.53	**Flufenacet metabolite M1**	**5000 µg**	14.67	3.06	1.42
	1500 µg	8.00	2.65	1.41		**1500 µg**	8.00	2.65	0.77
	500 µg	5.67	4.04	1.00		**500 µg**	7.33	4.04	0.71
	150 µg	8.00	2.00	1.41		**150 µg**	10.33	1.53	1.00
	50 µg	5.00	1.00	0.88		**50 µg**	11.33	2.08	1.10
Serie	**Dose/plate**	**Assay 2 (-S9 mix)**			**Serie**	**Dose/plate**	**Assay 2 with 10% S9-mix without preincubation**		
		Mean	**SD**	**R**			**Mean**	**SD**	**R**
Negative control	**100 µL**	5.67	6.35	-	**Negative control**	**100 µL**	8.00	3.61	-
Positive control solvent	**20 µL**	5.67	5.51	-	**Positive control solvent**	**10 µL**	7.00	5.29	-
Positive control: 9-Aminoacridine	**50µg in 20µL**	1805.33	169.35	318.59	**Positive control: 2-Anthramine**	**1µg in 10µL**	27.00	3.00	3.86
Vehicle	**50 µL**	8.00	4.00	-	**Vehicle**	**50 µL**	9.00	4.00	-
Flufenacet metabolite M1	**5000 µg**	10.67	4.51	1.33	**Flufenacet metabolite M1**	**5000 µg**	12.67	1.15	1.41
	1500 µg	7.67	0.58	0.96		**1500 µg**	14.67	3.21	1.63
	500 µg	10.00	1.00	1.25		**500 µg**	10.00	1.00	1.11
	150 µg	9.00	2.65	1.13		**150 µg**	12.00	2.65	1.33
	50 µg	9.00	4.36	1.13		**50 µg**	12.00	1.00	1.33

Mutagenic activity -TA98

Serie	**Dose/plate**	**Assay 1 (-S9 mix)**			**Serie**	**Dose/plate**	**Assay 1 with 10% S9-mix without preincubation**		
		Mean	**SD**	**R**			**Mean**	**SD**	**R**
Negative control	**100 µL**	13.67	4.73	-	**Negative control**	**100 µL**	16.33	3.06	-
Positive control solvent	**20 µL**	15.00	2.65	-	**Positive control solvent**	**20 µL**	20.67	5.51	-
Positive control: 2-Nitrofluorene	**2µg in 20µL**	205.00	17.78	13.67	**Positive control: 2-Anthramine**	**2µg in 20µL**	456.67	71.91	22.10
Vehicle	**50 µL**	10.67	2.89	-	**Vehicle**	**50 µL**	16.33	3.79	-
Flufenacet metabolite M1	**5000 µg**	10.67	2.08	1.00	**Flufenacet metabolite M1**	**5000 µg**	21.67	10.02	1.33
	1500 µg	10.33	2.08	0.97		**1500 µg**	20.00	2.65	1.22
	500 µg	12.67	0.58	1.19		**500 µg**	17.33	1.53	1.06
	150 µg	10.00	4.00	0.94		**150 µg**	20.33	1.53	1.24
	50 µg	12.00	2.00	1.13		**50 µg**	19.33	1.53	1.18
Serie	**Dose/plate**	**Assay 2 (-S9 mix)**			**Serie**	**Dose/plate**	**Assay 2 with 10% S9-mix without preincubation**		
		Mean	**SD**	**R**			**Mean**	**SD**	**R**
Negative	**100 µL**	11.33	1.53	-	**Negative**	**100 µL**	12.00	1.00	-

control					**control**				
Positive control solvent	**20 µL**	10.67	1.53	-	**Positive control solvent**	**10 µL**	12.00	1.00	-
Positive control: 2-Nitrofluorene	**2µg in 20µL**	203.33	18.88	19.06	**Positive control: 2-Anthramine**	**1µg in 10µL**	287.00	98.91	23.92
Vehicle	**50 µL**	8.67	3.79	-	**Vehicle**	**50 µL**	16.33	3.51	-
Flufenacet metabolite M1	**5000 µg**	9.00	2.65	1.04	**Flufenacet metabolite M1**	**5000 µg**	14.33	2.08	0.88
	1500 µg	13.67	3.06	1.58		**1500 µg**	12.00	1.73	0.73
	500 µg	9.00	2.00	1.04		**500 µg**	14.00	3.00	0.86
	150 µg	11.67	0.58	1.35		**150 µg**	14.00	2.00	0.86
	50 µg	14.33	1.15	1.65		**50 µg**	18.33	4.16	1.12

Mutagenic activity -TA100

Serie	**Dose/plate**	**Assay 1 (-S9 mix)**			**Serie**	**Dose/plate**	**Assay 1 with 10% S9-mix without preincubation**		
		Mean	**SD**	**R**			**Mean**	**SD**	**R**
Negative control	**100 µL**	63.00	17.52	-	**Negative control**	**100 µL**	80.33	3.51	-
Positive control solvent	**20 µL**	58.67	14.47	-	**Positive control solvent**	**20 µL**	76.67	6.03	-
Positive control: Sodium azide	**20µg in 20µL**	1317.67	27.50	22.46	**Positive control: 2-Anthramine**	**2µg in 20µL**	470.00	28.00	6.13
Vehicle	**50 µL**	49.67	8.02	-	**Vehicle**	**50 µL**	60.33	7.23	-
Flufenacet metabolite M1	**5000 µg**	50.00	7.00	1.01	**Flufenacet metabolite M1**	**5000 µg**	77.33	9.71	1.28
	1500 µg	53.33	5.51	1.07		**1500 µg**	74.33	5.13	1.23
	500 µg	46.67	2.08	0.94		**500 µg**	72.00	2.00	1.19
	150 µg	54.67	4.51	1.10		**150 µg**	75.00	5.29	1.24
	50 µg	59.67	9.29	1.20		**50 µg**	78.00	3.61	1.29
Serie	**Dose/plate**	**Assay 2 (-S9 mix)**			**Serie**	**Dose/plate**	**Assay 2 with 10% S9-mix without preincubation**		
		Mean	**SD**	**R**			**Mean**	**SD**	**R**
Negative control	**100 µL**	60.33	13.65	-	**Negative control**	**100 µL**	68.67	2.52	-
Positive control solvent	**20 µL**	56.67	4.73	-	**Positive control solvent**	**10 µL**	77.00	4.58	-
Positive control: Sodium azide	**20µg in 20µL**	1251.33	53.52	22.08	**Positive control: 2-Anthramine**	**1µg in 10µL**	401.67	11.06	5.22
Vehicle	**50 µL**	64.33	3.79	-	**Vehicle**	**50 µL**	71.67	5.51	-
Flufenacet metabolite M1	**5000 µg**	49.00	5.57	0.76	**Flufenacet metabolite M1**	**5000 µg**	80.33	8.62	1.12
	1500 µg	47.67	3.51	0.74		**1500 µg**	68.67	10.21	0.96
	500 µg	45.67	4.73	0.71		**500 µg**	77.00	13.53	1.07
	150 µg	48.00	6.08	0.75		**150 µg**	64.33	7.02	0.90
	50 µg	50.33	7.51	0.78		**50 µg**	78.33	3.51	1.09

Mutagenic activity -TA102

Serie	**Dose/plate**	**Assay 1 (-S9 mix)**	**Serie**	**Dose/plate**	**Assay 1 with 10% S9-**

							mix without preincubation		
		Mean	SD	R			Mean	SD	R
Negative control	**100 µL**	218.00	22.52	-	**Negative control**	**100 µL**	267.00	41.58	-
Positive control solvent	**31.25 µL**	230.33	18.61	-	**Positive control solvent**	**20 µL**	249.67	55.73	-
Positive control: Mitomycine C	**0.125µg in 31.25µL**	1203.67	43.14	5.23	**Positive control: Benzo (a) pyrene**	**2µg in 20µL**	792.33	21.39	3.17
Vehicle	**50 µL**	205.00	10.58	-	**Vehicle**	**50 µL**	286.67	24.09	-
Flufenacet metabolite M1	**5000 µg**	242.33	43.50	1.18	**Flufenacet metabolite M1**	**5000 µg**	311.33	18.93	1.09
	1500 µg	220.67	12.50	1.08		**1500 µg**	309.00	13.75	1.08
	500 µg	225.67	16.44	1.10		**500 µg**	285.67	40.53	1.00
	150 µg	226.67	11.68	1.11		**150 µg**	304.00	16.46	1.06
	50 µg	202.37	15.04	0.99		**50 µg**	278.33	32.32	0.97
Serie	**Dose/plate**	**Assay 2 (-S9 mix)**			**Serie**	**Dose/plate**	**Assay 2 with 10% S9-mix without preincubation**		
		Mean	**SD**	**R**			**Mean**	**SD**	**R**
Negative control	**100 µL**	181.67	27.59	-	**Negative control**	**100 µL**	312.67	1.53	-
Positive control solvent	**31.25 µL**	203.67	12.74	-	**Positive control solvent**	**20 µL**	219.67	36.77	-
Positive control: Mitomycine C	**0.125µg in 31.25µL**	741.00	90.76	3.64	**Positive control: Benzo (a) pyrene**	**2µg in 20µL**	584.33	12.90	2.66
Vehicle	**50 µL**	183.00	6.56	-	**Vehicle**	**50 µL**	191.67	10.97	-
Flufenacet metabolite M1	**5000 µg**	181.00	21.66	0.99	**Flufenacet metabolite M1**	**5000 µg**	304.67	12.42	1.59
	1500 µg	194.33	15.95	1.06		**1500 µg**	260.00	26.63	1.36
	500 µg	212.33	8.62	1.16		**500 µg**	257.33	52.55	1.34
	150 µg	204.00	10.58	1.11		**150 µg**	266.67	26.76	1.39
	50 µg	210.33	28.29	1.15		**50 µg**	223.33	22.72	1.17

Conclusions

Flufenacet metabolite M1 does not induce any mutagenic change in Salmonella typhimurium TA 1535, TA 1537, TA 98, TA 100 and TA 102 without or with metabolic activation.

Comments of zRMS:	The study conducted according to relevant OECD guidelines and in GLP principles and is acceptable. The test material is considered to be non-mutagenic in in vitro mammalian cell gene mutation test both with and without metabolic activation.

Reference	KCA 5.8.1
Report	In vitro mammalian cell gene mutation test with flufenacet metabolite M1. LEMI Laboratory. Study No.: 2015-FRU-5.
Guideline(s)	Yes, OECD 490
Deviations	No

GLP	Yes
Acceptability	Yes

Materials and methods

Flufenacet metabolite M1 was tested for its ability to induce chromosomal aberrations in cultured human lymphocytes. The study was carried out in the absence and presence of metabolic activation. Two independent experiments were performed.

For assay 1, lymphocytes were exposed to solutions of the test item in the absence of metabolic activation and 3hours in the presence of metabolic activation (S9mix 10% v/v). For assay 2, lymphocytes were exposed to solution of the test item 20h in absence of metabolic activation. For the two assays positive and negative controls were carried out in parallel.

Test item: flufenacet metabolite M1, purity 99.8%
Absolute negative control: Ham F12
Negative control (solvent): DMSO
Positive controls: without metabolic activation: Mitomycin C
With metabolic activation: Cyclophosphamide monohydrate
Assay conditions: before exposure, lymphocytes were stimulated 48h in the presence of 2% v/v phytohemagglutinin A (PHA). Cell cultures were incubated at 37°C in a humid atmosphere containing 5% v/v CO2.
Test item concentrations: 2000-800-320-128 µg/mL
Exposure of test item: Assay 1: without metabolic activation: 4h exposure followed by 18h expression
With metabolic activation (S9mix 10% v/v): 3h exposure followed by 18h Expression
Assay 2: with metabolic activation: 20h of exposition

Results

Survival – Assay (+S9-mix)

Survival							
Treatment		**EWs**	**TW**	**ns**	**c**	**PE survival**	**RCE**
Negative control		23	384	2	1.44	0.72	100%
		25					
		26					
		17					
Positive control: Cyclophosphamide monohydrate 2µg/L		42	192	2	0.90	0.45	63%
		36					
Solvent control: DMSO		16	384	2	1.63	0.82	100%
		18					
		24					
		17					
Flufenacet metabolite M1	2000µg/L	21	192	2	1.43	0.71	87%
		25					
	800µg/L	17	192	2	1.52	0.76	93%
		25					
	320µg/L	24	192	2	1.50	0.75	92%
		19					
	128µg/L	18	192	2	1.57	0.78	96%
		22					

EWs: Empty Wells for survival and viability plates at T48
TWs: Total Wells for survival and viability plates T48
ns: number of cells/well, plating cells at 10 cells per mL, i.e. 2 cells/well
c: probable number of clones/well= -ln(EWs/TWs)
PE: plating Efficiency survival (Pes)= c/ns

RCE: % (Pes dose/Pes solvent control) or (Pes positive control/Pes negative control)

Mutant frequency – Assay (+S9-mix)

Mutant frequency									
Treatment		**EWm**	**TWm**	**nm**	**PE mutant**	**(MF/survivors)x10^6**	**Induced mutants**	**X^2**	**P**
Negative control		165 163	384	2000	78.8	109.5	-	-	-
Positive control: Cyclophosphamide monohydrate 2µg/L		107 107	384	2000	292.3	649.1	539.6	84.8	<0.001
Solvent control: DMSO		157 156	384	2000	102.2	125.2	-	-	-
Flufenacet metabolite M1	2000µg/L	161 158	384	2000	92.7	129.8	4.6	0.0	NS
	800µg/L	143 146	384	2000	142.1	187.0	61.8	4.4	<0.05
	320µg/L	159 143	384	2000	120.1	160.5	35.4	1.6	NS
	128µg/L	154 154	384	2000	110.3	140.6	15.4	0.3	NS

EWm: Empty Wells plated for mutation to TFT resistance
TWm: Total Wells for mutation to TFT resistance
Nm: number of cells/well
PEmutants: $\text{PE mutants} = \left(-\ln\left(\frac{(EWm1+EWm2)}{TWm}\right) / nm\right) \times 10^{-6}$
MF: Mutant Frequency (MF/survivorsx10^6 =PEmutants/PEsurvival
Induced mutants= (MF/survivors dose – MF/suvivors solvent control) or (MF/survivors positive control – MF/Survisors negative control)
NS: not statistically significant if P≥0.05

Mutant frequency of the size of colonies (-S9-mix)

Treatment		**Σ EW small colonies**	**Σ EW large colonies**	**nm**	**Small colonies**		**Small colonies**	
					(MF/survivors) x10^6	**Induced mutants**	**(MF/survivors) x10^6**	**Induced mutants**
Negative control		344	341	2000	68	-	73	-
Positive control: NMS 10µg/L		215	289	2000	513	445	251	178
Solvent control: DMSO		352	339	2000	54	-	77	-
Flufenacet metabolite M1	2000µg/L	346	348	2000	87	33	82	5
	800µg/L	334	338	2000	101	47	92	15
	320µg/L	333	348	2000	84	30	58	-19
	128µg/L	346	330	2000	63	10	92	15

EWm: Empty Wells plated for mutation to TFT resistance
Nm: number of cells/well
MF: mutant frequency

Mutant frequency of the size of colonies (+S9-mix)

Treatment	**Σ EW small colonies**	**Σ EW large colonies**	**nm**	**Small colonies**		**Small colonies**	
				(MF/survivors) x10^6	**Induced mutants**	**(MF/survivors) x10^6**	**Induced mutants**
Negative control	367	345	2000	31	-	74	-
Positive control: Cyclophosphamide monohydrate 2µg/L	275	297	2000	371	339	285	211
Solvent control: DMSO	365	332	2000	31	-	89	-

Flufenacet metabolite M1	2000µg/L	351	351	2000	63	32	63	-26
	800µg/L	332	341	2000	96	65	78	-11
	320µg/L	345	341	2000	72	41	79	-10
	128µg/L	344	348	2000	70	39	63	-26

EWm: Empty Wells plated for mutation to TFT resistance
Nm: number of cells/well
MF: mutant frequency

Conclusions
In the framework of OECD 490 under the described experimental conditions, Flufenacet metabolite M1 does not induce a mutagenic effect in L5178Y TK+/-Mouse lymphoma cells in the absence or in the presence of metabolic activation (5% S9-mix)

Comments of zRMS:	The study conducted according to relevant OECD guidelines and in GLP principles is considered acceptable. Flufenacet metabolite M1 is considered to be non clastogenic for mammalian cells with and without metabolic activation in vitro.

Reference	KCA 5.8.1
Report	In vitro chromosome aberration in human lymphocytes of Flufeacet metabolite M1. LEMI Laboratory. Study No.: 2015-FRU-3.
Guideline(s)	Yes, OECD 473
Deviations	No
GLP	Yes
Acceptability	Yes

Materials and Methods

The flufenacet metabolite M1 was tested for its ability to induce chromosomal aberrations in cultured human lymphocytes. This study was carried out in the absence and presence of metabolic activation. Two independent experiments were performed.

For the first assay, lymphocyte were exposed to solutions of the test substance in the absence of metabolic activation and 3h in the presence of metabolic activation (S9-mix 10% v/v). For assay 2; lymphocytes were exposed to the test item 20h in absence of metabolic activation. For the two assays, positive and negative controls were carried out in parallel.

Test item: flufenacet metabolite M1, purity 99.8%
Absolute negative control: Ham F12
Negative control: DMSO
Positive controls: without metabolic activation: Mytomycin C
With metabolic activation: Cyclophosphamide monohydrate
Cell culture: before exposure to test item, lymphocytes are stimulated 48h in the presence of 2% v/v PHA and incubated at 37°C n a humid atmosphere containing 5% v/V CO_2.

Exposure concentrations used:
- Absolute negative control: culture medium, 10%, n=2
- Negative control (solvent): DMSO 0.67%, n=2
- Positive control without metabolic activation: Mytomycin C 0.25 µg/mL, n=2
- Positive control with metabolic activation: Cyclophosphamide monohydrate 50µg/mL, n=2

Results

Mitotic Index without Metabolic activation (-S9mix)

Series	Assay	Concentration	Cells observed	Cells in metaphase	Mitotic Index	Mitotix Index Reduction (%)
Negative control (culture medium)	1	-	1000	121	12.10	-
Positive control (Mitomycin C)		0.25 µg/mL	500	51	10.20	15.7
Solvent control (DMSO)		-	1000	101	10.10	-
Flufenacet metabolite M1		128 µg/mL	1000	94	9.40	6.9%
		320 µg/mL	1003	100	9.97	1.3%
		800 µg/mL	1057	109	10.31	-
		2000 µg/mL	1000	96	9.60	5.0%
Negative control (culture medium)	2	-	1004	130	12.95	-
Positive control (Mitomycin C)		0.25 µg/mL	503	36	7.16	44.7
Solvent control (DMSO)		-	1000	131	13.10	-
Flufenacet metabolite M2		128 µg/mL	1000	120	12.00	8.4
		320 µg/mL	1000	109	10.90	16.8
		800 µg/mL	1008	69	6.85	47.7
		2000 µg/mL	1012	44	4.35	66.8

Assay 1: 4 hours without metabolic activation; Assay 2: 20 hours without metabolic activation

Mitotic Index with Metabolic activation (+S9mix 10% (v/v))

Series	Assay	Concentration	Cells observed	Cells in metaphase	Mitotic Index	Mitotix Index Reduction (%)
Negative control (culture medium)	1	-	1000	88	8.80	-
Positive control (Cyclophosphamide)		50 µg/mL	500	24	4.80	45.5
Solvent control (DMSO)		-	1000	88	8.80	-
Flufenacet metabolite M2		128 µg/mL	1000	85	8.50	3.4
		320 µg/mL	1004	98	9.76	-
		800 µg/mL	1004	92	9.16	-
		2000 µg/mL	1077	76	7.06	19.8

Assay 1: 3 hours with metabolic activation S9 10% v/v

Chromosome aberrations in human lymphocytes in the absence of metabolic activation

Series	Assay	Concentration (µg/mL)	Cells observed	Aberrations (type and number)													
				Chromosomal						Chromatide						Other	
				G***	C	M	D	CR	R	g***	c	ic	tr	qr	d	CP	cp
Negative control (culture medium)	1	-	300	0	1	0	0	0	0	4	1	0	0	0	0	0	0
Positive		0.25	26	0	0	0	0	0	0	0	2	0	1	3	0	0	0

control (Mitomycin C)																	
Solvent control (DMSO)		-	302	1	1	0	0	0	0	3	2	0	0	0	0	0	0
Flufenacet metabolite M1		320	300	1	1	0	0	0	0	3	1	0	0	0	0	0	0
		800	300	0	2	0	0	0	0	3	1	0	0	0	0	0	0
		2000	300	0	1	0	0	0	0	5	4	0	1	1	0	0	0
Negative control (culture medium)	2	-	300	0	1	0	0	0	0	3	2	0	0	0	0	0	0
Positive control (Mitomycin C)		0.25	25	0	2	0	0	0	0	2	8	0	2	1	0	0	0
Solvent control (DMSO)		-	301	0	1	0	0	0	0	7	2	0	0	0	0	0	0
Flufenacet metabolite M1		128	300	0	1	0	0	0	0	1	1	0	0	0	0	0	0
		320	300	0	0	0	0	0	0	2	3	0	0	0	0	0	0
		800	300	0	2	0	0	0	0	4	5	0	0	0	0	0	0

Series	**Assay**	**Concentration**	**Cells observed**	**Total aberration**	**Aberration/cell**	**Cells with aberration**	**Cells with aberration (%)**	**X^2**	**p**
Negative control (culture medium)	1	-	300	2	0.007	2	0.67	-	-
Positive control (Mitomycin C)		0.25 µg/mL	26	6	0.231	6	23.08	50.20	<0.001
Solvent control (DMSO)		-	3002	3	0.010	3	0.99	-	-
Flufenacet metabolite M1		320 µg/mL	300	2	0.007	2	0.67	0.20	NS
		800 µg/mL	300	3	0.010	3	1.00	0.00	NS
		2000 µg/mL	300	7	0.023	6	2.00	1.04	NS
Negative control (culture medium)	2	-	300	3	1.010	3	1.00	-	-
Positive control (Mitomycin C)		0.25 µg/mL	25	13	0.520	12	48.00	115.79	<0.001
Solvent control (DMSO)		-	301	3	0.010	3	1.00	-	-
Flufenacet metabolite M2		128 µg/mL	300	2	0.007	2	0.67	0.20	NS
		320 µg/mL	300	3	0.010	3	1.00	1.00	NS
		800 µg/mL	300	7	0.023	7	2.33	1.65	NS

NS: not statistically significant, p≥0.05. Aberration of chromosome type: Gap or achromatic lesion (G**), Break or terminal deletion (C), Dicentric chromosome exchange (D or DF), comples rearrangement exchange (CR), ring exchange (R or RF), Minutes (M). Aberrations of chromatidic type: gap (g**), break ((c), meadian deletion (d), chromosome interchange (ci), triradial exchange (tr), quadriradial exchange (qr). Other events: pulverises chromosome (PC), pulverised cell (pc).

Chromosome aberrations in human lymphocytes in the presence of metabolic activation

Series	**Assay**	**Concentration (µg/mL)**	**Cells observed**	**Aberrations (type and number)**													
				Chromosomal						**Chromatide**						**Other**	
				G***	**C**	**M**	**D**	**CR**	**R**	**g*****	**c**	**ic**	**tr**	**qr**	**d**	**CP**	**cp**
Negative control (culture medium)	1	-	300	0	1	0	0	0	0	4	2	0	0	0	0	0	0
Positive		50	25	1	2	0	0	0	0	2	2	0	0	0	0	0	0

control (Cyclo-phospha-mide)																	
Solvent control (DMSO)		-	300	2	0	0	0	0	0	2	3	0	0	0	1	0	0
Flufenacet metabolite M2		320	300	0	0	0	0	0	0	3	2	0	0	0	0	0	0
		800	300	1	0	0	0	0	0	1	3	0	0	0	0	0	0
		2000	300	1	0	0	0	0	0	1	2	0	0	0	0	0	0

Series	**Assay**	**Concen-tration**	**Cells observed**	**Total ab-erration**	**Aberra-tion/cell**	**Cells with aberra-tion**	**Cells with aberra-tion (%)**	**X^2**	**P**
Negative control (culture medium)	1	-	300	3	0.010	3	1.00	-	-
Positive control (Cyclo-phospha-mide)		50 µg/mL	25	4	0.160	4	16.00	24.64	<0.001
Solvent control (DMSO)		-	300	4	0.013	4	1.33	-	-
Flufenacet metabolite M2		320 µg/mL	300	2	0.007	2	0.67	0.67	NS
		800 µg/mL	300	3	0.010	3	1.00	0.14	NS
		2000 µg/mL	300	2	0.007	2	0.67	0.67	NS

NS: not statistically significant, p≥0.05. Aberration of chromosome type: Gap or achromatic lesion (G**), Break or terminal deletion (C), Dicentric chromosome exchange (D or DF), comples rearrangement exchange (CR), ring exchange (R or RF), Minutes (M). Aberrations of chromatidic type: gap (g**), break ((c), meadian deletion (d), chromosome interchange (ci), triradial exchange (tr), quadriradial exchange (qr). Other events: pulverises chromosome (PC), pulverised cell (pc).

Conclusions

Flufenacet metabolite M1 is not considered clastogenic in the test system used (human lymphocytes) in the conditions of the assay.

Appendix 3 Exposure calculations

A 3.1 Operator exposure calculations (KCP 7.2.1.1)

A 3.1.1 Calculations for Aclonifen

Table A 1: Input parameters considered for the estimation of operator exposure

Operator exposure for Aclonifen outdoor spray applications

Application rate of active substance	1.08 kg a.s./ha	*i_AppRate*
Assumed area treated	50 ha/day	*d_AreaTreated*
Amount of active substance applied	54 kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	0.04%	*i_AbsorpProduct*
Dermal absorption of in-use dilution	2.10%	*i_AbsorInuse*
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.	
Indoor or Outdoor application	Outdoor	
Application method	Downward spraying	
Application equipment	Vehicle-mounted	
Season	not relevant	

OutdoorSoluble concentrates, emulsifiable concentrate, etc.Downward sprayingVehicle-mounted

	Exposure values	µg exposure/day mixed and loaded 75th centile	95th centile	Reference	Comment
Mixing and loading	Hands	104715	397440	AOEM	
	Body	58895	229499	AOEM	
	Head	2802	15366	AOEM	
	Protected hands (gloves)	462	10696	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	816	7898	AOEM	
	Protected head (hood and face shield)	45	870	AOEM	
	Inhalation	12	32	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied 75th centile	95th centile	Reference	Comment
Application	Hands	8009	42559	AOEM	
	Body	4478	23086	AOEM	
	Head	212	638	AOEM	
	Protected hands (gloves)	370	5307	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	123	301	AOEM	
	Inhalation	8	29	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Closed cab	No		vehicle mounted upward spraying only	

Table A 2: Estimation of longer term operator exposure towards active substance according to EFSA guidance

1. Total

	Without RPE/PPE	With RPE/PPE
Longer term		
Total systemic exposure from mixing, loading and application (mg a.s./day)	0.3580197	0.2415796
Total systemic exposure from mixing, loading and application per kg body weight (mg/kg bw/day)	0.0059670	0.0040263
% of RVNAS	8.52%	5.75%

Table A 3: Input parameters considered for the estimation of operator exposure (gloves during mixing/loading)

Operator exposure for Aclonifen outdoor spray applications

Application rate of active substance	1.08	kg a.s./ha	*i_AppRate*
Assumed area treated	50	ha/day	*d_AreaTreated*
Amount of active substance applied	54	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	0.04%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	2.10%		*i_AbsorInuse*
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.		
Indoor or Outdoor application	Outdoor		
Application method	Downward spraying		
Application equipment	Vehicle-mounted		
Season	not relevant		

Mixing and loading

Exposure values	µg exposure/day mixed and loaded		Reference	Comment
	75th centile	95th centile		
Hands	104715	397440	AOEM	
Body	58895	229499	AOEM	
Head	2802	15366	AOEM	
Protected hands (gloves)	462	10696	AOEM	
Protected body (workwear or protective garment and sturdy footwear)	816	7898	AOEM	
Protected head (hood and face shield)	45	870	AOEM	
Inhalation	12	32	AOEM	
Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
Gloves	Yes		Incl. in AOEM model	
Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
Head and respiratory PPE	None		1	1
Water soluble bag	No		1	

Application

Exposure values	µg exposure/day applied		Reference	Comment
	75th centile	95th centile		
Hands	8009	42559	AOEM	
Body	4478	23086	AOEM	
Head	212	638	AOEM	
Protected hands (gloves)	370	5307	AOEM	
Protected body (workwear or protective garment and sturdy footwear)	123	301	AOEM	
Inhalation	8	29	AOEM	
Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
Gloves	No			
Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
Head and respiratory PPE	None		1	1
Closed cab	No		vehicle mounted upward spraying only	

Table A 4: Estimation of longer term operator exposure towards active substance according to EFSA guidance (gloves during mixing/loading)

1. Total

	Without RPE/PPE	With RPE/PPE
Longer term		
Total systemic exposure from mixing, loading and application (mg a.s./day)	0.3580197	0.1967508
Total systemic exposure from mixing, loading and application per kg body weight (mg/kg bw/day)	0.0059670	0.0032792
% of RVNAS	8.52%	4.68%

A 3.1.2 Calculations for Flufenacet

Table A 5: Input parameters considered for the estimation of operator exposure

Operator exposure for Flufenacet outdoor spray applications

Application rate of active substance	0.12 kg a.s./ha	*i_AppRate*	
Assumed area treated	50 ha/day	*d_AreaTreated*	
Amount of active substance applied	6 kg a.s./day	*i_AmoutAS*	
Dermal absorption of the product	10.00%	*i_AbsorpProduct*	
Dermal absorption of in-use dilution	50.00%	*i_AbsorInuse*	
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.		
Indoor or Outdoor application	Outdoor		
Application method	Downward spraying		
Application equipment	Vehicle-mounted		
Season	not relevant		

	Exposure values	µg exposure/day mixed and loaded		Reference	Comment
		75th centile	95th centile		
Mixing and loading	Hands	19293	71819	AOEM	
	Body	12569	121213	AOEM	
	Head	311	1707	AOEM	
	Protected hands (gloves)	111	1188	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	116	878	AOEM	
	Protected head (hood and face shield)	5	97	AOEM	
	Inhalation	6	30	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied		Reference	Comment
		75th centile	95th centile		
Application	Hands	890	8513	AOEM	
	Body	498	2565	AOEM	
	Head	24	71	AOEM	
	Protected hands (gloves)	112	4107	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	14	33	AOEM	
	Inhalation	3	8	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Closed cab	Yes		vehicle mounted upward spraying only	

Table A 6: Estimation of longer term operator exposure towards active substance according to EFSA guidance

1. Total

	Without RPE/PPE	With RPE/PPE
Longer term		
Total systemic exposure from mixing, loading and application (mg a.s./day)	**3.9317712**	**2.4444881**
Total systemic exposure from mixing, loading and application per kg body weight (mg/kg bw/day)	**0.0655295**	**0.0407415**
% of RVNAS	**385.47%**	**239.66%**

Table A 7: Input parameters considered for the estimation of operator exposure (gloves during mixing/loading)

Operator exposure for Flufenacet outdoor spray applications

Application rate of active substance	0.12	kg a.s./ha	*i_AppRate*
Assumed area treated	50	ha/day	*d_AreaTreated*
Amount of active substance applied	6	kg a.s./day	*i_AmoutAS*
Dermal absorption of the product	10.00%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	50.00%		*i_AbsorInuse*
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.		
Indoor or Outdoor application	Outdoor		
Application method	Downward spraying		
Application equipment	Vehicle-mounted		
Season	not relevant		

	Exposure values	µg exposure/day mixed and loaded 75th centile	µg exposure/day mixed and loaded 95th centile	Reference	Comment
Mixing and loading	Hands	19293	71819	AOEM	
	Body	12569	121213	AOEM	
	Head	311	1707	AOEM	
	Protected hands (gloves)	111	1188	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	116	878	AOEM	
	Protected head (hood and face shield)	5	97	AOEM	
	Inhalation	6	30	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	Yes		Incl. in AOEM model	
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Water soluble bag	No		1	

	Exposure values	µg exposure/day applied 75th centile	µg exposure/day applied 95th centile	Reference	Comment
Application	Hands	890	8513	AOEM	
	Body	498	2565	AOEM	
	Head	24	71	AOEM	
	Protected hands (gloves)	112	4107	AOEM	
	Protected body (workwear or protective garment and sturdy footwear)	14	33	AOEM	
	Inhalation	3	8	AOEM	
	Protective Equipment	Select for inclusion		Penetration factor	Inhalation Protection factor
	Gloves	No			
	Clothing	Work wear - arms, body and legs covered		Incl. in AOEM model	
	Head and respiratory PPE	None		1	1
	Closed cab	No		vehicle mounted upward spraying only	

Table A 8: Estimation of longer term operator exposure towards active substance according to EFSA guidance (gloves during mixing/loading)

1. Total

	Without RPE/PPE	With RPE/PPE
Longer term		
Total systemic exposure from mixing, loading and application (mg a.s./day)	**3.9317712**	**0.5262201**
Total systemic exposure from mixing, loading and application per kg body weight (mg/kg bw/day)	**0.0655295**	**0.0087703**
% of RVNAS	**385.47%**	**51.59%**

A 3.2 Worker exposure calculations (KCP 7.2.3.1)

A 3.2.1 Calculations for Aclonifen

Table A 9: Input parameters considered for the estimation of worker exposure

Worker exposure from residues on foliage for Aclonifen		
Crop type	**Cereals**	
Indoor or outdoor	**Outdoor**	
Application method	**Downward spraying**	
Application equipment	**Vehicle-mounted**	
Worker's task	**Inspection, irrigation**	
Main body parts in contact with foliage	**Hand and body**	
Application rate of active substance	**1.08**	kg a.s./ha
Number of applications	**1**	
Interval between multiple applications	**365**	days
Half-life of active substance	**30**	days
Multiple application factor	**1.0**	
Dermal absorption of the product	**0.04%**	
Dermal absorption of the in-use dilution	**2.10%**	
Dislodgeable foliar residue (i_AppRate*i_DFR)	**3.24**	µg a.s./cm^2
Working hours	**2**	hr
Dermal transfer coefficient - Total potential exposure	**12500**	cm^2/hr
Dermal transfer coefficient - arms, body and legs covered	**1400**	cm^2/hr
Dermal transfer coefficient - hands, arms, body and legs covered	**no TC available for this assessment**	cm^2/hr
Inhalation transfer coefficient for automated applications	**NA**	ha/hr*10^(-3)
Inhalation transfer coefficient for cutting ornamentals	**NA**	ha/hr*10^(-3)
Inhalation transfer coefficient for sorting / bundling ornamentals	**NA**	ha/hr*10^(-3)

Table A 10: Estimation of longer term worker exposure towards Aclonifen according to EFSA guidance

1. Total

	Potential exposure	Work wear - arms, body and legs covered	Working wear and gloves
Total systemic exposure (mg a.s./day)	**1.7010000**	**0.1905120**	**no TC available for this assessment**
Total systemic exposure per kg body weight (mg/kg bw/day)	**0.0283500**	**0.0031752**	
% of RVNAS	**40.50%**	**4.54%**	

A 3.2.2 Calculations for flufenacet

Table A 11: Input parameters considered for the estimation of worker exposure

Worker exposure from residues on foliage for Flufenacet		
Crop type	Cereals	
Indoor or outdoor	Outdoor	
Application method	Downward spraying	
Application equipment	Vehicle-mounted	
Worker's task	Inspection, irrigation	
Main body parts in contact with foliage	Hand and body	
Application rate of active substance	0.12	kg a.s./ha
Number of applications	1	
Interval between multiple applications	365	days
Half-life of active substance	30	days
Multiple application factor	1.0	
Dermal absorption of the product	10.00%	
Dermal absorption of the in-use dilution	50.00%	
Dislodgeable foliar residue (i_AppRate*i_DFR)	0.36	µg a.s./cm^2
Working hours	2	hr
Dermal transfer coefficient - Total potential exposure	12500	cm^2/hr
Dermal transfer coefficient - arms, body and legs covered	1400	cm^2/hr
Dermal transfer coefficient - hands, arms, body and legs covered	no TC available for this assessment	cm^2/hr
Inhalation transfer coefficient for automated applications	NA	ha/hr*10^(-3)
Inhalation transfer coefficient for cutting ornamentals	NA	ha/hr*10^(-3)
Inhalation transfer coefficient for sorting / bundling ornamentals	NA	ha/hr*10^(-3)

Table A 12: Estimation of longer term worker exposure towards flufenacet according to EFSA guidance

1. Total			
	Potential exposure	Work wear - arms, body and legs covered	Working wear and gloves
Total systemic exposure (mg a.s./day)	4.5000000	0.5040000	no TC available for this assessment
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0750000	0.0084000	
% of RVNAS	441.18%	49.41%	

A 3.3 Resident and bystander exposure calculations (KCP 7.2.2.1)

A 3.3.1 Calculations for Aclonifen

Table A 13: Input parameters considered for the estimation of longer term resident exposure (2L formulation/ha, 200 L water/ha)

Resident exposure for glosset			
Croptype	Cereals		
Application method	Downward spraying		
Application equipment	Vehicle-mounted		i_AppEquip
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.		i_FormVal
Buffer strip	2-3	m	i_Buffer
Application rate of the product	1,08	kg a.s./ha	i_AppRate
Concentration of active substance (in-use dilution for liquid applications)	5,4	g a.s./l	d_ConcAS
Dermal absorption of product	0,04%		i_AbsorpProduct
Dermal absorption of in-use dilution	2,10%		i_AbsorpInuse
Oral absorption	100,00%		i_AbsorpOralInuse
Dislodgeable foliar residue (i_AppRate*i_DFR)	3,24	µg a.s./cm^2	d_DFR
Vapour pressure of in-use dilution	low volatile substances having a vapour pressure of <5*10-3Pa	Pa	i_Volat
Concentration in air	0,001	mg/m^3	d_AirCon
Resident dermal spray drift exposure 75th percentile - adult	0,47	ml spray dilution/person	
Resident dermal spray drift exposure 75th percentile - child	0,327	ml spray dilution/person	
Resident inhal. spray drift exposure 75th percentile - adult	0,00010	ml spray dilution/person	
Resident inhal. spray drift exposure 75th percentile - child	0,00022	ml spray dilution/person	
Resident dermal spray drift exposure mean - adult	0,22318	ml spray dilution/person	
Resident dermal spray drift exposure mean - child	0,18	ml spray dilution/person	
Resident inhal. spray drift exposure mean - adult	0,00009	ml spray dilution/person	
Resident inhal. spray drift exposure mean - child	0,00017	ml spray dilution/person	
Exposure duration dermal	2	hours	d_ReExpDur
Exposure duration inhalation	24	hours	d_ReExpDurInhal
Exposure duration entry into treated crops	0,25	hours	d_ExpDurTreatCrop
Light clothing adjustment factor	18,0%		d_ClothAF
Breathing rate adult	0,23	m^3/day/kg	d_BreathRAd
Breathing rate child (1-3 year old)	1,07	m^3/day/kg	d_BreathRCh
Drift percentage on surface (75th percentile)	5,60%		
Drift percentage on surface (mean)	4,10%		
Turf transferable residues percentage	5,00%		d_Turf
Transfer coeff. of surface deposits-adult	7300	cm^2/hour	d_ReTCAd
Transfer coeff. of surface deposits-child (1-3 year old)	2600	cm^2/hour	d_ReTCCh
Saliva extraction percentage	50,00%		d_SalExt
Surface area of hands mouthed	20	cm^2	d_AreaHM
Frequency of hand to mouth activity	9,5	events/hour	d_ReFreqHM
Ingestion rate for mouthing of grass per day	25	cm^2	d_MouthGrass
Dislodgeable residues percentage transferability for object to mouth	20,00%		d_DRP
Transfer coefficient for entry into treated crops (75th percentile) - ad	7500	cm^2/h	d_TcEntryAd
Transfer coefficient for entry into treated crops (75th percentile) - chi	2250	cm^2/h	d_TcEntryCh
Transfer coefficient for entry into treated crops (mean) - adult	5980	cm^2/h	d_TcEntryAd
Transfer coefficient for entry into treated crops (mean) - child	1794	cm^2/h	d_TcEntryCh

Table A 14: Estimation of longer term resident exposure towards Aclonifen according to EFSA guidance (2L formulation/ha, 200 L water/ha)

1. Total

1.1 1-3 year old child

	Spray drift (75th percentile)	Vapour (75th percentile)	Surface deposits (75th percentile)	Entry into treated crops (75th percentile)	All pathways (mean)
Total systemic exposure (mg a.s./day)	0,0315951	0,0107000	0,0120718	0,0382725	0,0677101
Total systemic exposure per kg body weight (mg/kg bw/day)	0,0031595	0,0010700	0,0012072	0,0038273	0,0067710
% of RVNAS	4,51%	1,53%	1,72%	5,47%	9,67%

1.2 Adult

	Spray drift	Vapour	Surface deposits	Entry into treated crops	All pathways (mean)
Total systemic exposure (mg a.s./day)	0,0442444	0,0138000	0,0092716	0,1275750	0,1435470
Total systemic exposure per kg body weight	0,0007374	0,0002300	0,0001545	0,0021263	0,0023924
% of RVNAS	1,05%	0,33%	0,22%	3,04%	3,42%

Table A 15: Input parameters considered for the estimation of longer term resident exposure (2L formulation/ha, 300 L water/ha)

Resident exposure for Glosset Ace			
Croptype	Cereals		
Application method	Downward spraying		
Application equipment	Vehicle-mounted		*i_AppEquip*
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.		*i_FormVal*
Buffer strip	2-3	m	*i_Buffer*
Application rate of the product	1,08	kg a.s./ha	*i_AppRate*
Concentration of active substance (in-use dilution for liquid applications)	3,6	g a.s./l	*d_ConcAS*
Dermal absorption of product	0,04%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	2,10%		*i_AbsorpInuse*
Oral absorption	100,00%		*i_AbsorpOralInuse*
Dislodgeable foliar residue (i_AppRate*i_DFR)	3,24	µg a.s./cm^2	*d_DFR*
Vapour pressure of in-use dilution	low volatile substances having a vapour pressure of <5*10-3Pa	Pa	*i_Volat*
Concentration in air	0,001	mg/m^3	*d_AirCon*
Resident dermal spray drift exposure 75th percentile - adult	0,47	ml spray dilution/person	
Resident dermal spray drift exposure 75th percentile - child	0,327	ml spray dilution/person	
Resident inhal. spray drift exposure 75th percentile - adult	0,00010	ml spray dilution/person	
Resident inhal. spray drift exposure 75th percentile - child	0,00022	ml spray dilution/person	
Resident dermal spray drift exposure mean - adult	0,22318	ml spray dilution/person	
Resident dermal spray drift exposure mean - child	0,18	ml spray dilution/person	
Resident inhal. spray drift exposure mean - adult	0,00009	ml spray dilution/person	
Resident inhal. spray drift exposure mean - child	0,00017	ml spray dilution/person	
Exposure duration dermal	2	hours	*d_ReExpDur*
Exposure duration inhalation	24	hours	*d_ReExpDurInhal*
Exposure duration entry into treated crops	0,25	hours	*d_ExpDurTreatCrop*
Light clothing adjustment factor	18,0%		*d_ClothAF*
Breathing rate adult	0,23	m^3/day/kg	*d_BreathRAd*
Breathing rate child (1-3 year old)	1,07	m^3/day/kg	*d_BreathRCh*
Drift percentage on surface (75th percentile)	5,60%		
Drift percentage on surface (mean)	4,10%		
Turf transferable residues percentage	5,00%		*d_Turf*
Transfer coeff. of surface deposits-adult	7300	cm^2/hour	*d_ReTCAd*
Transfer coeff. of surface deposits-child (1-3 year old)	2600	cm^2/hour	*d_ReTCCh*
Saliva extraction percentage	50,00%		*d_SalExt*
Surface area of hands mouthed	20	cm^2	*d_AreaHM*
Frequency of hand to mouth activity	9,5	events/hour	*d_ReFreqHM*
Ingestion rate for mouthing of grass per day	25	cm^2	*d_MouthGrass*
Dislodgeable residues percentage transferability for object to mouth	20,00%		*d_DRP*
Transfer coefficient for entry into treated crops (75th percentile) - ad	7500	cm^2/h	*d_TcEntryAd*
Transfer coefficient for entry into treated crops (75th percentile) - chi	2250	cm^2/h	*d_TcEntryCh*
Transfer coefficient for entry into treated crops (mean) - adult	5980	cm^2/h	*d_TcEntryAd*
Transfer coefficient for entry into treated crops (mean) - child	1794	cm^2/h	*d_TcEntryCh*

Table A 16: Estimation of longer term resident exposure towards Aclonifen according to EFSA guidance (2L formulation/ha, 300 L water/ha)

1. Total

1.1 1-3 year old child

	Spray drift (75th percentile)	Vapour (75th percentile)	Surface deposits (75th percentile)	Entry into treated crops (75th percentile)	All pathways (mean)
Total systemic exposure (mg a.s./day)	0,0210634	0,0107000	0,0120718	0,0382725	0,0618248
Total systemic exposure per kg body weight (mg/kg bw/day)	0,0021063	0,0010700	0,0012072	0,0038273	0,0061825
% of RVNAS	3,01%	1,53%	1,72%	5,47%	8,83%

1.2 Adult

	Spray drift	Vapour	Surface deposits	Entry into treated crops	All pathways (mean)
Total systemic exposure (mg a.s./day)	0,0294962	0,0138000	0,0092716	0,1275750	0,1364673
Total systemic exposure per kg body weight	0,0004916	0,0002300	0,0001545	0,0021263	0,0022745
% of RVNAS	0,70%	0,33%	0,22%	3,04%	3,25%

Table A 17: Input parameters considered for the estimation of longer term resident exposure (1.5L formulation/ha, 150 L water/ha)

Resident exposure for Glosset Ace - aclonifen				
Croptype	Cereals			
Application method	Downward spraying			
Application equipment	Vehicle-mounted			*i_AppEquip*
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.			*i_FormVal*
Buffer strip	2-3	m		*i_Buffer*
Application rate of the product	0,81	kg a.s./ha		*i_AppRate*
Concentration of active substance (in-use dilution for liquid applications)	5,4	g a.s./l		*d_ConcAS*
Dermal absorption of product	0,04%			*i_AbsorpProduct*
Dermal absorption of in-use dilution	2,10%			*i_AbsorpInuse*
Oral absorption	100,00%			*i_AbsorpOralInuse*
Dislodgeable foliar residue (i_AppRate*i_DFR)	2,43	µg a.s./cm^2		*d_DFR*
Vapour pressure of in-use dilution	low volatile substances having a vapour pressure of <5*10-3Pa	Pa		*i_Volat*
Concentration in air	0,001	mg/m^3		*d_AirCon*
Resident dermal spray drift exposure 75th percentile - adult	0,47	ml spray dilution/person		
Resident dermal spray drift exposure 75th percentile - child	0,327	ml spray dilution/person		
Resident inhal. spray drift exposure 75th percentile - adult	0,00010	ml spray dilution/person		
Resident inhal. spray drift exposure 75th percentile - child	0,00022	ml spray dilution/person		
Resident dermal spray drift exposure mean - adult	0,22318	ml spray dilution/person		
Resident dermal spray drift exposure mean - child	0,18	ml spray dilution/person		
Resident inhal. spray drift exposure mean - adult	0,00009	ml spray dilution/person		
Resident inhal. spray drift exposure mean - child	0,00017	ml spray dilution/person		
Exposure duration dermal	2	hours		*d_ReExpDur*
Exposure duration inhalation	24	hours		*d_ReExpDurInhal*
Exposure duration entry into treated crops	0,25	hours		*d_ExpDurTreatCrop*
Light clothing adjustment factor	18,0%			*d_ClothAF*
Breathing rate adult	0,23	m^3/day/kg		*d_BreathRAd*
Breathing rate child (1-3 year old)	1,07	m^3/day/kg		*d_BreathRCh*
Drift percentage on surface (75th percentile)	5,60%			
Drift percentage on surface (mean)	4,10%			
Turf transferable residues percentage	5,00%			*d_Turf*
Transfer coeff. of surface deposits-adult	7300	cm^2/hour		*d_ReTCAd*
Transfer coeff. of surface deposits-child (1-3 year old)	2600	cm^2/hour		*d_ReTCCh*
Saliva extraction percentage	50,00%			*d_SalExt*
Surface area of hands mouthed	20	cm^2		*d_AreaHM*
Frequency of hand to mouth activity	9,5	events/hour		*d_ReFreqHM*
Ingestion rate for mouthing of grass per day	25	cm^2		*d_MouthGrass*
Dislodgeable residues percentage transferability for object to mouth	20,00%			*d_DRP*
Transfer coefficient for entry into treated crops (75th percentile) - ad	7500	cm^2/h		*d_TcEntryAd*
Transfer coefficient for entry into treated crops (75th percentile) - chi	2250	cm^2/h		*d_TcEntryCh*
Transfer coefficient for entry into treated crops (mean) - adult	5980	cm^2/h		*d_TcEntryAd*
Transfer coefficient for entry into treated crops (mean) - child	1794	cm^2/h		*d_TcEntryCh*

Table A 18: Estimation of longer term resident exposure towards Aclonifen according to EFSA guidance (1.5L formulation/ha, 150 L water/ha)

1. Total

1.1 1-3 year old child

	Spray drift (75th percentile)	Vapour (75th percentile)	Surface deposits (75th percentile)	Entry into treated crops (75th percentile)	All pathways (mean)
Total systemic exposure (mg a.s./day)	0,0315951	0,0107000	0,0090539	0,0287044	0,0578715
Total systemic exposure per kg body weight (mg/kg bw/day)	0,0031595	0,0010700	0,0009054	0,0028704	0,0057872
% of RVNAS	4,51%	1,53%	1,29%	4,10%	8,27%

1.2 Adult

	Spray drift	Vapour	Surface deposits	Entry into treated crops	All pathways (mean)
Total systemic exposure (mg a.s./day)	0,0442444	0,0138000	0,0069537	0,0956813	0,1164200
Total systemic exposure per kg body weight	0,0007374	0,0002300	0,0001159	0,0015947	0,0019403
% of RVNAS	1,05%	0,33%	0,17%	2,28%	2,77%

Table A 19: Input parameters considered for the estimation of longer term resident exposure (1.5L formulation/ha, 300 L water/ha)

Resident exposure for Aclonifen		
Croptype	Cereals	
Application method	Downward spraying	
Application equipment	Vehicle-mounted	
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.	
Buffer strip	2-3	m
Application rate of the product	0.81	kg a.s./ha
Concentration of active substance (in-use dilution for liquid applications)	2.7	g a.s./l
Dermal absorption of product	0.04%	
Dermal absorption of in-use dilution	2.10%	
Oral absorption	100.00%	
Dislodgeable foliar residue (i_AppRate*i_DFR)	2.43	µg a.s./cm^2
Vapour pressure of in-use dilution	low volatile substances having a vapour pressure of <5*10-3Pa	Pa
Concentration in air	0.001	mg/m^3
Resident dermal spray drift exposure 75th percentile - adult	0.47	ml spray dilution/person
Resident dermal spray drift exposure 75th percentile - child	0.327	ml spray dilution/person
Resident inhal. spray drift exposure 75th percentile - adult	0.00010	ml spray dilution/person
Resident inhal. spray drift exposure 75th percentile - child	0.00022	ml spray dilution/person
Resident dermal spray drift exposure mean - adult	0.22318	ml spray dilution/person
Resident dermal spray drift exposure mean - child	0.18	ml spray dilution/person
Resident inhal. spray drift exposure mean - adult	0.00009	ml spray dilution/person
Resident inhal. spray drift exposure mean - child	0.00017	ml spray dilution/person
Exposure duration dermal	2	hours
Exposure duration inhalation	24	hours
Exposure duration entry into treated crops	0.25	hours
Light clothing adjustment factor	18.0%	
Breathing rate adult	0.23	m^3/day/kg
Breathing rate child (1-3 year old)	1.07	m^3/day/kg
Drift percentage on surface (75th percentile)	5.60%	
Drift percentage on surface (mean)	4.10%	
Turf transferable residues percentage	5.00%	
Transfer coeff. of surface deposits-adult	7300	cm^2/hour
Transfer coeff. of surface deposits-child (1-3 year old)	2600	cm^2/hour
Saliva extraction percentage	50.00%	
Surface area of hands mouthed	20	cm^2
Frequency of hand to mouth activity	9.5	events/hour
Ingestion rate for mouthing of grass per day	25	cm^2
Dislodgeable residues percentage transferability for object to mouth	20.00%	
Transfer coefficient for entry into treated crops (75th percentile) - adult	7500	cm^2/h
Transfer coefficient for entry into treated crops (75th percentile) - child	2250	cm^2/h
Transfer coefficient for entry into treated crops (mean) - adult	5980	cm^2/h
Transfer coefficient for entry into treated crops (mean) - child	1794	cm^2/h

Table A 20: Estimation of longer term resident exposure towards Aclonifen according to EFSA guidance (1.5L formulation/ha, 300 L water/ha)

1. Total

1.1 1-3 year old child

	Spray drift (75th percentile)	Vapour (75th percentile)	Surface deposits (75th percentile)	Entry into treated crops (75th percentile)	All pathways (mean)
Total systemic exposure (mg a.s./day)	0.0157975	0.0107000	0.0090539	0.0287044	0.0490436
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0015798	0.0010700	0.0009054	0.0028704	0.0049044
% of RVNAS	2.26%	1.53%	1.29%	4.10%	7.01%

1.2 Adult

	Spray drift	Vapour	Surface deposits	Entry into treated crops	All pathways (mean)
Total systemic exposure (mg a.s./day)	0.0221222	0.0138000	0.0069537	0.0956813	0.1058005
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0003687	0.0002300	0.0001159	0.0015947	0.0017633
% of RVNAS	0.53%	0.33%	0.17%	2.28%	2.52%

A 3.3.2 Calculations for flufenacet

Table A 21: Input parameters considered for the estimation of longer term resident exposure (2L formulation/ha, 200 L water/ha)

Resident exposure for Flufenacet			
Croptype	Cereals		
Application method	Downward spraying		
Application equipment	Vehicle-mounted		*i_AppEquip*
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.		*i_FormVal*
Buffer strip	2-3	m	*i_Buffer*
Application rate of the product	0.12	kg a.s./ha	*i_AppRate*
Concentration of active substance (in-use dilution for liquid applications)	0.6	g a.s./l	*d_ConcAS*
Dermal absorption of product	10.00%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	50.00%		*i_AbsorpInuse*
Oral absorption	100.00%		*i_AbsorpOralInuse*
Dislodgeable foliar residue (i_AppRate*i_DFR)	0.36	µg a.s./cm^2	*d_DFR*
Vapour pressure of in-use dilution	low volatile substances having a vapour pressure of <5*10-3Pa	Pa	*i_Volat*
Concentration in air	0.001	mg/m^3	*d_AirCon*
Resident dermal spray drift exposure 75th percentile - adult	0.47	ml spray dilution/person	
Resident dermal spray drift exposure 75th percentile - child	0.327	ml spray dilution/person	
Resident inhal. spray drift exposure 75th percentile - adult	0.00010	ml spray dilution/person	
Resident inhal. spray drift exposure 75th percentile - child	0.00022	ml spray dilution/person	
Resident dermal spray drift exposure mean - adult	0.22318	ml spray dilution/person	
Resident dermal spray drift exposure mean - child	0.18	ml spray dilution/person	
Resident inhal. spray drift exposure mean - adult	0.00009	ml spray dilution/person	
Resident inhal. spray drift exposure mean - child	0.00017	ml spray dilution/person	
Exposure duration dermal	2	hours	*d_ReExpDur*
Exposure duration inhalation	24	hours	*d_ReExpDurInhal*
Exposure duration entry into treated crops	0.25	hours	*d_ExpDurTreatCrop*
Light clothing adjustment factor	18.0%		*d_ClothAF*
Breathing rate adult	0.23	m^3/day/kg	*d_BreathRAd*
Breathing rate child (1-3 year old)	1.07	m^3/day/kg	*d_BreathRCh*
Drift percentage on surface (75th percentile)	5.60%		
Drift percentage on surface (mean)	4.10%		
Turf transferable residues percentage	5.00%		*d_Turf*
Transfer coeff. of surface deposits-adult	7300	cm^2/hour	*d_ReTCAd*
Transfer coeff. of surface deposits-child (1-3 year old)	2600	cm^2/hour	*d_ReTCCh*
Saliva extraction percentage	50.00%		*d_SalExt*
Surface area of hands mouthed	20	cm^2	*d_AreaHM*
Frequency of hand to mouth activity	9.5	events/hour	*d_ReFreqHM*
Ingestion rate for mouthing of grass per day	25	cm^2	*d_MouthGrass*
Dislodgeable residues percentage transferability for object to mouth	20.00%		*d_DRP*
Transfer coefficient for entry into treated crops (75th percentile) - adult	7500	cm^2/h	*d_TcEntryAd*
Transfer coefficient for entry into treated crops (75th percentile) - child	2250	cm^2/h	*d_TcEntryCh*
Transfer coefficient for entry into treated crops (mean) - adult	5980	cm^2/h	*d_TcEntryAd*
Transfer coefficient for entry into treated crops (mean) - child	1794	cm^2/h	*d_TcEntryCh*

Table A 22: Estimation of longer term resident exposure towards flufenacet according to EFSA guidance ((2L formulation/ha, 200 L water/ha)

1. Total

1.1 1-3 year old child

	Spray drift (75th percentile)	Vapour (75th percentile)	Surface deposits (75th percentile)	Entry into treated crops (75th percentile)	All pathways (mean)
Total systemic exposure (mg a.s./day)	0.0805740	0.0107000	0.0097104	0.1012500	0.1429214
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0080574	0.0010700	0.0009710	0.0101250	0.0142921
% of RVNAS	47.40%	6.29%	5.71%	59.56%	84.07%

1.2 Adult

	Spray drift	Vapour	Surface deposits	Entry into treated crops	All pathways (mean)
Total systemic exposure (mg a.s./day)	0.1156800	0.0138000	0.0245280	0.3375000	0.3558143
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0019280	0.0002300	0.0004088	0.0056250	0.0059302
% of RVNAS	11.34%	1.35%	2.40%	33.09%	34.88%

Table A 23: Input parameters considered for the estimation of longer term resident exposure (2L formulation/ha, 300Lwater/ha)

Resident exposure for Flufenacet		
Croptype	Cereals	
Application method	Downward spraying	
Application equipment	Vehicle-mounted	
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.	
Buffer strip	2-3	m
Application rate of the product	0.12	kg a.s./ha
Concentration of active substance (in-use dilution for liquid applications)	0.4	g a.s./l
Dermal absorption of product	10.00%	
Dermal absorption of in-use dilution	50.00%	
Oral absorption	100.00%	
Dislodgeable foliar residue (i_AppRate*i_DFR)	0.36	µg a.s./cm^2
Vapour pressure of in-use dilution	low volatile substances having a vapour pressure of <5*10-3Pa	Pa
Concentration in air	0.001	mg/m^3
Resident dermal spray drift exposure 75th percentile - adult	0.47	ml spray dilution/person
Resident dermal spray drift exposure 75th percentile - child	0.327	ml spray dilution/person
Resident inhal. spray drift exposure 75th percentile - adult	0.00010	ml spray dilution/person
Resident inhal. spray drift exposure 75th percentile - child	0.00022	ml spray dilution/person
Resident dermal spray drift exposure mean - adult	0.22318	ml spray dilution/person
Resident dermal spray drift exposure mean - child	0.18	ml spray dilution/person
Resident inhal. spray drift exposure mean - adult	0.00009	ml spray dilution/person
Resident inhal. spray drift exposure mean - child	0.00017	ml spray dilution/person
Exposure duration dermal	2	hours
Exposure duration inhalation	24	hours
Exposure duration entry into treated crops	0.25	hours
Light clothing adjustment factor	18.0%	
Breathing rate adult	0.23	m^3/day/kg
Breathing rate child (1-3 year old)	1.07	m^3/day/kg
Drift percentage on surface (75th percentile)	5.60%	
Drift percentage on surface (mean)	4.10%	
Turf transferable residues percentage	5.00%	
Transfer coeff. of surface deposits-adult	7300	cm^2/hour
Transfer coeff. of surface deposits-child (1-3 year old)	2600	cm^2/hour
Saliva extraction percentage	50.00%	
Surface area of hands mouthed	20	cm^2
Frequency of hand to mouth activity	9.5	events/hour
Ingestion rate for mouthing of grass per day	25	cm^2
Dislodgeable residues percentage transferability for object to mouth	20.00%	
Transfer coefficient for entry into treated crops (75th percentile) - adult	7500	cm^2/h
Transfer coefficient for entry into treated crops (75th percentile) - child	2250	cm^2/h
Transfer coefficient for entry into treated crops (mean) - adult	5980	cm^2/h
Transfer coefficient for entry into treated crops (mean) - child	1794	cm^2/h

Table A 24: Estimation of longer term resident exposure towards flufenacet according to EFSA guidance (2L formulation/ha, 300Lwater/ha)

1. Total

1.1 1-3 year old child

	Spray drift (75th percentile)	Vapour (75th percentile)	Surface deposits (75th percentile)	Entry into treated crops (75th percentile)	All pathways (mean)
Total systemic exposure (mg a.s./day)	0.0537160	0.0107000	0.0097104	0.1012500	0.1281274
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0053716	0.0010700	0.0009710	0.0101250	0.0128127
% of RVNAS	31.60%	6.29%	5.71%	59.56%	75.37%

1.2 Adult

	Spray drift	Vapour	Surface deposits	Entry into treated crops	All pathways (mean)
Total systemic exposure (mg a.s./day)	0.0771200	0.0138000	0.0245280	0.3375000	0.3374955
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0012853	0.0002300	0.0004088	0.0056250	0.0056249
% of RVNAS	7.56%	1.35%	2.40%	33.09%	33.09%

Table A 25: Input parameters considered for the estimation of longer term resident exposure (1.5L formulation/ha, 150Lwater/ha)

Resident exposure for Flufenacet			
Croptype	Cereals		
Application method	Downward spraying		
Application equipment	Vehicle-mounted-Drift Reduction		*i_AppEquip*
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.		*i_FormVal*
Buffer strip	2-3	m	*i_Buffer*
Application rate of the product	0.09	kg a.s./ha	*i_AppRate*
Concentration of active substance (in-use dilution for liquid applications)	0.6	g a.s./l	*d_ConcAS*
Dermal absorption of product	10.00%		*i_AbsorpProduct*
Dermal absorption of in-use dilution	50.00%		*i_AbsorpInuse*
Oral absorption	100.00%		*i_AbsorpOralInuse*
Dislodgeable foliar residue (i_AppRate*i_DFR)	0.27	µg a.s./cm^2	*d_DFR*
Vapour pressure of in-use dilution	low volatile substances having a vapour pressure of <5*10-3Pa	Pa	*i_Volat*
Concentration in air	0.001	mg/m^3	*d_AirCon*
Resident dermal spray drift exposure 75th percentile - adult	0.47	ml spray dilution/person	
Resident dermal spray drift exposure 75th percentile - child	0.327	ml spray dilution/person	
Resident inhal. spray drift exposure 75th percentile - adult	0.00010	ml spray dilution/person	
Resident inhal. spray drift exposure 75th percentile - child	0.00022	ml spray dilution/person	
Resident dermal spray drift exposure mean - adult	0.22318	ml spray dilution/person	
Resident dermal spray drift exposure mean - child	0.18	ml spray dilution/person	
Resident inhal. spray drift exposure mean - adult	0.00009	ml spray dilution/person	
Resident inhal. spray drift exposure mean - child	0.00017	ml spray dilution/person	
Exposure duration dermal	2	hours	*d_ReExpDur*
Exposure duration inhalation	24	hours	*d_ReExpDurInhal*
Exposure duration entry into treated crops	0.25	hours	*d_ExpDurTreatCrop*
Light clothing adjustment factor	18.0%		*d_ClothAF*
Breathing rate adult	0.23	m^3/day/kg	*d_BreathRAd*
Breathing rate child (1-3 year old)	1.07	m^3/day/kg	*d_BreathRCh*
Drift percentage on surface (75th percentile)	5.60%		
Drift percentage on surface (mean)	4.10%		
Turf transferable residues percentage	5.00%		*d_Turf*
Transfer coeff. of surface deposits-adult	7300	cm^2/hour	*d_ReTCAd*
Transfer coeff. of surface deposits-child (1-3 year old)	2600	cm^2/hour	*d_ReTCCh*
Saliva extraction percentage	50.00%		*d_SalExt*
Surface area of hands mouthed	20	cm^2	*d_AreaHM*
Frequency of hand to mouth activity	9.5	events/hour	*d_ReFreqHM*
Ingestion rate for mouthing of grass per day	25	cm^2	*d_MouthGrass*
Dislodgeable residues percentage transferability for object to mouth	20.00%		*d_DRP*
Transfer coefficient for entry into treated crops (75th percentile) - adult	7500	cm^2/h	*d_TcEntryAd*
Transfer coefficient for entry into treated crops (75th percentile) - child	2250	cm^2/h	*d_TcEntryCh*
Transfer coefficient for entry into treated crops (mean) - adult	5980	cm^2/h	*d_TcEntryAd*
Transfer coefficient for entry into treated crops (mean) - child	1794	cm^2/h	*d_TcEntryCh*

Table A 26: Estimation of longer term resident exposure towards flufenacet according to EFSA guidance (1.5L formulation/ha, 150Lwater/ha)

1. Total

1.1 1-3 year old child

	Spray drift (75th percentile)	Vapour (75th percentile)	Surface deposits (75th percentile)	Entry into treated crops (75th percentile)	All pathways (mean)
Total systemic exposure (mg a.s./day)	0.0402870	0.0107000	0.0036414	0.0759375	0.0961045
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0040287	0.0010700	0.0003641	0.0075938	0.0096105
% of RVNAS	23.70%	6.29%	2.14%	44.67%	56.53%

1.2 Adult

	Spray drift	Vapour	Surface deposits	Entry into treated crops	All pathways (mean)
Total systemic exposure (mg a.s./day)	0.0578400	0.0138000	0.0091980	0.2531250	0.2498374
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0009640	0.0002300	0.0001533	0.0042188	0.0041640
% of RVNAS	5.67%	1.35%	0.90%	24.82%	24.49%

Table A 27: Input parameters considered for the estimation of longer term resident exposure (1.5L formulation/ha, 300Lwater/ha)

Resident exposure for Flufenacet		
Croptype	Cereals	
Application method	Downward spraying	
Application equipment	Vehicle-mounted	
Formulation type	Soluble concentrates, emulsifiable concentrate, etc.	
Buffer strip	2-3	m
Application rate of the product	0.09	kg a.s./ha
Concentration of active substance (in-use dilution for liquid applications)	0.3	g a.s./l
Dermal absorption of product	10.00%	
Dermal absorption of in-use dilution	50.00%	
Oral absorption	100.00%	
Dislodgeable foliar residue (i_AppRate*i_DFR)	0.27	µg a.s./cm^2
Vapour pressure of in-use dilution	low volatile substances having a vapour pressure of <5*10-3Pa	Pa
Concentration in air	0.001	mg/m^3
Resident dermal spray drift exposure 75th percentile - adult	0.47	ml spray dilution/person
Resident dermal spray drift exposure 75th percentile - child	0.327	ml spray dilution/person
Resident inhal. spray drift exposure 75th percentile - adult	0.00010	ml spray dilution/person
Resident inhal. spray drift exposure 75th percentile - child	0.00022	ml spray dilution/person
Resident dermal spray drift exposure mean - adult	0.22318	ml spray dilution/person
Resident dermal spray drift exposure mean - child	0.18	ml spray dilution/person
Resident inhal. spray drift exposure mean - adult	0.00009	ml spray dilution/person
Resident inhal. spray drift exposure mean - child	0.00017	ml spray dilution/person
Exposure duration dermal	2	hours
Exposure duration inhalation	24	hours
Exposure duration entry into treated crops	0.25	hours
Light clothing adjustment factor	18.0%	
Breathing rate adult	0.23	m^3/day/kg
Breathing rate child (1-3 year old)	1.07	m^3/day/kg
Drift percentage on surface (75th percentile)	5.60%	
Drift percentage on surface (mean)	4.10%	
Turf transferable residues percentage	5.00%	
Transfer coeff. of surface deposits-adult	7300	cm^2/hour
Transfer coeff. of surface deposits-child (1-3 year old)	2600	cm^2/hour
Saliva extraction percentage	50.00%	
Surface area of hands mouthed	20	cm^2
Frequency of hand to mouth activity	9.5	events/hour
Ingestion rate for mouthing of grass per day	25	cm^2
Dislodgeable residues percentage transferability for object to mouth	20.00%	
Transfer coefficient for entry into treated crops (75th percentile) - adult	7500	cm^2/h
Transfer coefficient for entry into treated crops (75th percentile) - child	2250	cm^2/h
Transfer coefficient for entry into treated crops (mean) - adult	5980	cm^2/h
Transfer coefficient for entry into treated crops (mean) - child	1794	cm^2/h

Table A 28: Estimation of longer term resident exposure towards flufenacet according to EFSA guidance (1.5L formulation/ha, 300Lwater/ha)

1. Total

1.1 1-3 year old child

	Spray drift (75th percentile)	Vapour (75th percentile)	Surface deposits (75th percentile)	Entry into treated crops (75th percentile)	All pathways (mean)
Total systemic exposure (mg a.s./day)	0.0402870	0.0107000	0.0072828	0.0759375	0.0987706
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0040287	0.0010700	0.0007283	0.0075938	0.0098771
% of RVNAS	23.70%	6.29%	4.28%	44.67%	58.10%

1.2 Adult

	Spray drift	Vapour	Surface deposits	Entry into treated crops	All pathways (mean)
Total systemic exposure (mg a.s./day)	0.0578400	0.0138000	0.0183960	0.2531250	0.2565716
Total systemic exposure per kg body weight (mg/kg bw/day)	0.0009640	0.0002300	0.0003066	0.0042188	0.0042762
% of RVNAS	5.67%	1.35%	1.80%	24.82%	25.15%

Appendix 4 Detailed evaluation of exposure and/or DFR studies relied upon (KCP 7.2, KCP 7.2.1.1, KCP 7.2.2.1, KCP 7.2.3.1)

No DFR or exposure studies were submitted within the frame of this application.